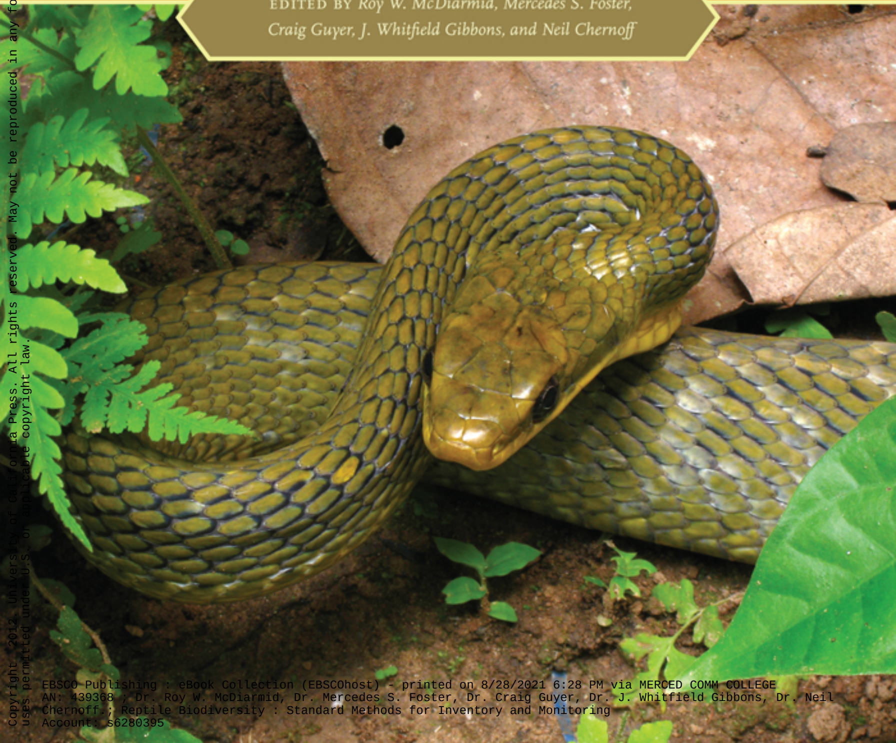
REPTILE BIODIVERSITY

STANDARD METHODS *for* INVENTORY *and* MONITORING

EDITED BY *Roy W. McDiarmid, Mercedes S. Foster, Craig Guyer, J. Whitfield Gibbons, and Neil Chernoff*



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UNIVERSITY OF CALIFORNIA PRESS
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FOREWORD

RICK SHINE

This is the book that I desperately needed at the beginning of my scientific career. Like most other young herpetologists, I had a pretty simple set of ideas about how to gather data—I'd just go out there, look for snakes, find some, catch them, and then write down anything that seemed useful (such as their sex or body size). And somehow or other, once I'd been doing that for long enough, I'd have a data set that could then tell all kinds of interesting stories about the biology of the creatures in question. Fortunately, there were wiser heads around—notably my doctoral supervisor, Hal Heatwole, who not only is still an active researcher but also has contributed to this volume. And after talking with those more experienced herpetologists, it dawned on me that I actually needed to think about what I was going to do, and how I might ultimately use the data, *before* I started my fieldwork.

One of the glories of fieldwork is serendipity: you never really know what you might discover (famously, Albert Einstein commented that if we knew what we were doing, it wouldn't be research). My own career has been full of such surprises: I've been astonished by some peculiar facet or other in the biology of almost every species that I have studied. At first sight, a standardizing of methods—one of the main points of this book—might seem counter to that spirit of free inquiry. It's not. If your data set is gathered with a robust sampling design and in a way that can be compared directly to other data sets gathered at other places and other times on the same or different species, the opportunities to discover something genuinely new and exciting are magnified a thousand-fold. And it is only by bringing separately gathered data sets together that we can address many issues that are critical for conservation and management. For example, some of the most important challenges to the viability of reptile populations—such as climate change or the overall impact of invasive species or diseases—operate at timescales and spatial scales too large for any single field study.

Field-based surveys on any organisms need robust sampling designs as well as methods that are logistically feasible and ethically acceptable. But the need is greater—and the challenge greater—for reptiles than for many other types of animals. As any phylogenetic purist will tell you, there's no such thing as a "reptile" in evolutionary terms—squamates (lizards and snakes) haven't shared a common ancestor with turtles, crocodiles, or tuataras for a very long time, and these independent evolutionary histories have produced animals that differ from each other in many ways. Even within each of the major lineages, reptiles are amazingly diverse in almost every aspect. One inevitable result is that an equally diverse array of methods is needed for studies of those animals—for example, the techniques that enable one to most effectively find, collect, and study a blind snake embryo differ in substantial ways from the techniques needed to investigate adult saltwater crocodiles. That diversity creates an enormous challenge for anyone who attempts a book like the current volume. Fortunately, the critical importance of the task has enabled the editors to find a stellar list of contributors, including many of the world's most experienced field herpetologists.

The task is urgent. In many parts of the world, reptile populations are being menaced by a cocktail of threatening processes, ultimately brought about by human activities. Reptiles are not unique in their vulnerability to threats such as habitat degradation, climate change, and invasive species—but the central role of reptiles in many ecosystems is rarely appreciated. Popular attention tends to focus on endothermic vertebrates, especially large mammals and brightly colored birds. If we look to the tropics, however—the parts of the world that are the most biodiverse and that are subject to the most intense threats—the diversity and abundance of ectothermic ("cold-blooded") vertebrates like reptiles and amphibians vastly exceeds the numbers and types of birds and mammals. Our current estimates of reptile biodiversity in most tropical regions also are far too low, with new species being described every month. The small body sizes, low metabolic rates, and sedentary behaviors of many reptile species have resulted in many taxa being restricted to very small areas, some of which undoubtedly have been destroyed long before the species within them were ever seen by scientists. As ectotherms, reptiles rely upon ambient temperature regimes for many aspects of their day-to-day lives, and climate change may pose a massive challenge to the continued existence of many species.

Reptilian ectothermy also poses a huge challenge to any attempt to establish and describe standard techniques for biodiversity monitoring. The intimate dependence of reptile behavior on local weather conditions means that surveys conducted at different times can yield massively different numbers of animals, and the ability to remain inactive and unfed for weeks or months at a time makes these animals a nightmare to survey quantitatively—a high proportion of the population may be virtually inaccessible to an observer for most of the year. The reptiles of severely seasonal climatic regimes offer an obvious example: calculating abundances of garter snakes on the Manitoba prairies is impossible for the 8 months per year that they spend underground, before emerging during the brief warm period. But many examples are less obvious: for example, pit-vipers on the small island of Shedao in northern China are active only for a few weeks each year in spring and autumn, because these are the only times that their main prey—migrating passerine birds—pass through the island in seasonal migrations. Any attempt to monitor abundances or population attributes in such systems must incorporate knowledge of their ecology, and hence of the appropriate periods for sampling. Species' peculiarities like this don't make it impossible to apply standard techniques for biodiversity monitoring, but they do place a premium on refining methods in light of local conditions and ecological circumstances.

In summary, then, monitoring reptile populations in standardized, repeatable ways is not an easy task—but it's a critically important one. Unless we can accurately measure what's out there in the wild, we cannot detect how, when, where, and why things are going wrong. There will never be a simple "one size fits all" formula for asking questions of nature, but standardization—and utilization of the most powerful sampling designs—can add enormously to the value of the information that is collected. This book, encapsulating the hard-won experience of expert field herpetologists, offers many ideas and protocols that will improve the quality of our science and ultimately help us to conserve the organisms that we cherish.

Rick Shine
University of Sydney
May 2010

PREFACE

Historical Perspective

This volume is the fourth in a series of handbooks developed to identify and present standard methods for the inventory and monitoring of biological diversity. Ground-level information about biodiversity provides the basis for informed decisions about the protection, sustainable use, or development of specific areas or species and for the rehabilitation or restoration of degraded environments. Yet our knowledge of biodiversity, including the geographical and habitat distributions of most organisms, ranges from imprecise and incomplete to nonexistent, and many habitats and biotas are disappearing or being degraded faster than their biodiversity can be inventoried. Funds for such work tend to be scarce, and the number of trained scientists, technicians, and managers is limited, particularly in the least-explored areas of developing countries. By collecting and recording data according to standard methods and protocols and analyzing them in standard ways, the utility of the data that are being gathered is maximized. Data from diverse studies can be compared across sites and through time; they can also be combined to provide regional or even larger-scale coverage. Given the added value of standardized data, it is surprising that no generally accepted and widely used standard methods for sampling the diversity of most organisms are available.

This lack was particularly evident when suspicions of global declines in amphibian populations arose in the late 1980s. Verification of supposed population changes was hampered by the absence of quantitative baseline population data against which to compare current population levels. In a (belated) attempt to fill that void, Mercedes Foster initiated the Biodiversity Methods Handbooks project in 1990. She assembled a group of amphibian biologists and encouraged them to produce a volume identifying, detailing the use of, and advocating a series of standardized methods for inventorying and monitoring the biodiversity of amphibians. The anticipated book would cover biodiversity sampling in all habitats and ecosystems wherever amphibians might occur throughout the world. It would also include sections on project design, data analysis, data and specimen standards, GIS, and similar topics generally applicable to all biodiversity studies. Having the standard protocols in a single volume in a user-friendly format would save time and funds in the planning of any inventory or monitoring project because it would not be necessary for investigators to “reinvent the wheel.” In addition, a user-friendly handbook would be readily available for training field practitioners, technicians, and students.

With the broad participation and cooperation of the community of amphibian biologists, the first volume in the series (Heyer et al. 1994a), focusing on Amphibians, was produced. Duplicating the model, volumes on standardized methods for use with mammals and fungi followed. The present volume deals with reptiles, a group that represents a challenge for many reasons, including the highly disparate morphologies, ecologies, and behaviors found among extant groups and the scarcity of systematic, quantitative baseline data against which to measure population changes that may have occurred over time. Yet reptile populations face a multitude of threats, including habitat destruction, overcollecting for the pet trade, excess and often unregulated commercial harvest for food, skins, or trinkets, incidental anthropogenic mortality, and general animosity toward some groups (e.g., poisonous snakes) that leads to their wanton killing. Consequently, when Neil Chernoff approached Mercedes Foster and Roy McDiarmid about the possibility of producing a handbook dealing with methods for reptiles, their responses were extremely enthusiastic.

Foster convened a core committee (Roy McDiarmid, Craig Guyer, and Neil Chernoff) to develop a preliminary plan for the book and seek comments and expressions of interest from the greater community of reptile biologists. The committee subsequently invited individuals who had experience with the inventory and monitoring of reptiles to attend a workshop to complete the outline for the book and assume responsibility for preparing chapters. The workshop was held from 29 to 31 August 2002 in Arlington, Virginia. Participants included Neil Chernoff, Robert Fisher, Lee Fitzgerald, Mercedes Foster, Whit Gibbons, Craig Guyer, Lee-Ann Hayek, John Lee, Robert Lovitch, Roy McDiarmid (Workshop Chair), Joe Mitchell, Jim Nichols, Charles Peterson, Christopher Raxworthy, Robert Reed, and Gordon Rodda. At that time, McDiarmid, Foster, Guyer, Gibbons, and Chernoff assumed responsibility for compiling and editing the publication.

Despite the best of intentions, completion of the manuscript was delayed because of illness, lack of funds, and author delays. It was finally sent to the Press on 19 December 2010. Ultimately, 70 reptile biologists and others with overwhelming collective expertise and from six countries contributed to the volume. The participation of so many highly experienced individuals helped to ensure that the book provides comprehensive and up-to-date coverage of sampling methodologies and data analyses, and we believe that the volume will make a major contribution to the study of reptile biodiversity. However, sampling methods and analytical procedures are never static. We encourage investigators interested in reptilian biodiversity to refine and improve the methods covered in this book, and we look forward to the development of new and innovative procedures.

Acknowledgments

Many people worked tirelessly to bring this ambitious project to completion, and we offer them our heartfelt thanks. We are especially grateful to Paul Ustach for providing the delightful cartoons that liven up the volume. We also thank all those field herpetologists who generously allowed us to use their photographs and authors and publishers who provided permission to reprint previously published material.

Lynda J. Garrett of the USGS Patuxent Wildlife Research Center (USGS/PWRC) Library worked miracles in locating and obtaining copies of reports from the gray literature, Federal and State documents, and mainstream publications. Patricia L. Lasker, Martha A. Rosen, and David T. Steere, Jr., of the Smithsonian Institution's National Museum of Natural History Branch Library also gave generously of their time to obtain copies of publications. Fiona Wilkinson (USGS/PWRC) helped with the verification of literature references and supplier contact information, prepared several of the figures, and assisted Foster with a myriad of miscellaneous tasks related to preparation of the manuscript. James D. Nichols (USGS/PWRC) and Sally T. Murphy contributed in other ways.

We are deeply indebted to the authors for contributing the manuscripts that make up this volume and greatly appreciate their willingness to participate in the project. We are especially grateful to the participants who submitted their manuscripts on time and maintained their equanimity in the face of delays caused by those who did not. Finally, we would like to thank Chuck Crumly, our Editor at the University of California Press, for his infinite patience during the preparation of this volume and his faith in our ability to finish the manuscript. We also thank Lynn Meinhardt and Francisco Reinking, our Editorial Coordinator and Project Editor at the Press, respectively, and Edward Wade, Production Editor at Westchester Book Services, all of whom answered our questions and provided extremely helpful advice and direction.

The Environmental Protection Agency provided generous financial support during the early stages of this project, including funding for the workshop. Other financial and administrative support was provided by the USGS Patuxent Wildlife Research Center and the Smithsonian Institution's National Museum of Natural History. Any royalties from the book will be donated to Partners in Amphibian and Reptile Conservation (PARC) to support its national and international activities (http://www.parcplace.org/about.html).

The Editors
Washington, D. C.
December 19, 2010

PART ONE

INTRODUCTION

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CHAPTER ONE

Studying Reptile Diversity

MERCEDES S. FOSTER, ROY W. McDIARMID, and NEIL CHERNOFF

> Biological diversity is the key to the maintenance of the world as we know it.
>
> E. O. WILSON, 1992

> Sentient species that we are, *Homo sapiens* is able to understand the shape, extent, and value of Earth's biodiversity. Indeed, we have a responsibility, as well as a self-interest, to value it.
>
> RICHARD E. LEAKEY and ROGER LEWIN, 1995

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Setting the Stage

This volume is the fourth in a series of publications dealing with standard methods for inventorying and monitoring the biodiversity of different taxa (e.g., see Heyer et al. 1994a; Wilson et al. 1996; Mueller et al. 2004). The impetus for the first volume was the sudden realization in the late 1980s and early 1990s that amphibian populations were declining globally, for unknown reasons. Determining the scope (numbers of species affected) and the magnitude of the declines was hampered by the absence of baseline data with which to compare contemporary population levels and by the lack of comparability among the data sets that were available.

About the time that the amphibian declines were first being recognized, there were dramatic surges in interest and concern about the worldwide loss of biodiversity, even though various individuals and institutions had been sounding the alarm for decades. In 1986, the National Academy of Sciences and the Smithsonian Institution sponsored the National Forum on BioDiversity, held in Washington, DC, on September 21–24, and published the proceedings of that forum (see Wilson 1988). In 1992 (3–14 June), the United Nations held the Conference on Environment and Development (UNCED) in Rio de Janeiro, Brazil. The attendees, who numbered between 20,000 and 30,000, represented the governments of 178 countries as well as nongovernmental organizations (NGOs) and the news media. One product of that meeting was *The United Nations Convention on Biological Diversity* (http://www.cbd.int/convention/text/), often referred to as the Biodiversity Treaty. Recurring themes of the conference and the Convention were that countries needed to conserve their biological diversity and use their biological resources in a sustainable manner. At a meeting in April 2002, the Conference of the Parties (the decision-making body of the Convention) set as a goal a significant reduction in the rate of loss of biodiversity, which they hoped might be achieved by 2010, the United Nations–designated International Year of Biodiversity. They recognized, however, that in most instances the available taxonomic information was too limited to form a basis for decisions about the conservation and sustainable use of our living resources. To help reduce this "taxonomic impediment," they established the Global Taxonomy Initiative to train taxonomists, expand scientific collecting, and increase the pace of taxonomic work, particularly the description of new species (SCBD 2008).

After the Rio meeting, many countries rose to the challenge, designating new protected areas, developing environmental education programs, and increasing efforts to manage exploited and threatened ecosystems. In addition, countries, companies, and conservationists began placing more emphasis on sustainable development and habitat restoration rather than just on the protection of pristine areas, but at the same time recognizing the importance of ecosystem services (carbon sequestration, clean air and water, soil maintenance, pollinator populations and food production, sources of raw materials with newly recognized uses) whose economic value has been largely unacknowledged. Nevertheless, by the time you read this the 2010 deadline will have passed, and although rates have perhaps slowed, habitats continue to shrink, species continue to decline or go extinct (Hoffman et al. 2010; Marton-Lefèvre 2010), and government and conservation groups continue to base decisions regarding conservation, management, and development on woefully inadequate and incomplete data about the distributions and population statuses of species. At a time when our need to use our resources more efficiently and maintain healthy populations of the species and ecosystems of greatest value to the health of the planet, we still do not know the species composition of most ecosystems, understand the function of each in those ecosystems and how they maintain it, or appreciate the impact the loss of any one of them might have. Without such information, conserving biodiversity, restoring

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degraded habitats, and reconstituting ecosystems will be impossible.

Some 25 years after recognizing the global decline of amphibian populations, biologists and conservationists are becoming increasingly aware of declines in reptile populations (Gibbons et al. 2000; Yardley 2007; Sinervo 2010). And once again, we are faced with an absence of baseline data with which to make comparisons. Nearly 9,100 species of reptiles are known, and new taxa continue to be described at a prodigious rate. Uetz (2010) estimated that on average nearly 100 new species of reptiles were described each year between 1998 and 2008, a rate higher than that for any 10-year interval since 1758, and it continues to accelerate. Reptiles make up more than half of the biomass in some ecosystems and must play a significant role in ecosystem function. Yet, we have no knowledge of many components of their basic natural history. The lack of data may in part be attributable to the less charismatic nature (e.g., compared to birds and mammals) of these organisms that may even engender fear and hostility on the part of some—there is no “Kermit” equivalent to speak for them.

The herpetofaunas of many areas of Europe and much of eastern North America have been studied for more than 150 years. Regional summaries published from the late 19th to mid-20th centuries were followed by field guides to the species of most temperate regions. The guides included drawings of the species accompanied by descriptions and geographic distributions. Until quite recently there have not been field guides or regional summaries to reptiles in most tropical regions, although species descriptions are available and distributions are moderately well known. The basic components of their ecologies, in contrast, are not. For example, in a recent comprehensive and well-illustrated field guide to the reptiles of Southeast Asia, Das (2010) included in each species account a section titled “Habitat and Behavior.” More frequently than not, these sections refer to diet, reproductive behavior, and other natural history traits with notations such as “unknown,” “nothing known,” and “unstudied.” A review of the accounts revealed that 288 (67%) of 428 species of lizards in 11 families were data deficient, with values ranging from 0 percent for a few small, well-studied families (e.g., Lanthanotidae and Varanidae) to highs of 84 and 93 percent, respectively, for species in the poorly know families Anguidae and Dibamidae. Similarly, 260 (54%) of 482 species of snakes in 12 families were data deficient, ranging from 0 percent for snakes in the families Pythonidae and Xenopeltidae to 59 and 82 percent for species in the families Colubridae and Typhlopidae, respectively. Not surprisingly, venomous species are moderately well studied, with only about 40 percent of the species in both the Elapidae and the Viperidae being data-deficient. Values for lizards and snakes throughout the Neotropics are likely similar (RWM, pers. observ.). Thus, while field guides are very useful for introducing the reptiles of an area to both professionals and amateurs, the existence of such books in no way signals that knowledge of the animals’ biologies is even adequate. That belief, the “field guide syndrome,” rarely reflects reality. Even in parts of the world where reptiles have been well studied, basic knowledge of the natural history of most species is poorly known, and knowledge gaps are rarely reported in field guides. Given the increasing rates of discovery of new taxa, the better sampling of poorly known geographic areas, and the increasing number of local herpetologists carrying out quality research on reptiles in Asia, Africa, and the Americas, progress is being made. Recent publications reflect those advances (e.g., Spawls et al. 2002; Carreira et al. 2005; Das 2010). We hope that this volume will contribute to that effort.

In this volume we provide information on carrying out standardized quantitative surveys of reptile biodiversity that can be compared across habitats and through time. We include chapters addressing issues to consider when planning a study, selecting sampling techniques, performing a survey, and analyzing the data obtained. It is our fervent hope that this volume will motivate scientists, managers, conservationists, and nature lovers, whether employed by governments or conservation groups or simply aficionados of reptiles, to carry out surveys of the biodiversity of this poorly know but exceedingly important group.

Importance of Standardization

Throughout this book we advocate the use of standard methods for inventory and monitoring. Standard methods increase the comparability of data and decrease the effects of variables extraneous to a study. Nevertheless, because reptile species exhibit such a wide range of morphologies, behaviors, and ecologies, it is impossible to identify a single technique that will sample all members of a reptile community equally. Species differ, including closely related forms, and so do groups within species (e.g., males and females, juveniles and adults, and reproductive and nonreproductive individuals). How many and which techniques will be employed in a study will depend largely on its intent and whether it focuses on a single species, members of a particular behavioral or ecological guild, or all members of a community. And although a single species may be sampled with more than one technique, studies of whole communities will require the use of several. Obviously, techniques appropriate for aquatic turtles will be less than relevant for sampling arboreal lizards. In fact, we encourage the use of more than one technique when time, personnel, and other resources permit.

It is possible to sample many groups of reptiles quite appropriately with several different techniques or variations of a single technique. And most field biologists have favorites developed by trial and error in the field or learned at their mentor’s knee. We are not suggesting that the techniques we have identified are the only appropriate ones or even the very best for a particular group. Rather, we have selected those that we believe can best be standardized, are easiest to use with the least likelihood of error, provide the greatest return for the effort and resources expended, and yet produce scientifically sound and statistically rigorous data. However, when comparing different sites or different times, even employing a standard sampling technique is insufficient if investigators do not adhere strictly to the indicated protocol, something that we strongly encourage. We also recommend that investigators record detailed *metadata* (i.e., data about the data), including not only when and how the primary data were collected but also the locations of study sites and when and how to access them; the distribution and nature of habitats and other environmental features within those sites that are important for reptiles (maps and photographs are very useful); data on weather conditions at the time of collecting; descriptions of training provided to technicians, assistants, and volunteers; and lists of vouchers (specimens, photographs, videos, etc.) collected and where they are deposited. Such information significantly increases the value of the primary data and enables

WE NEED TO GET ON THE SAME PAGE WITH THIS "BIPEDALISM" THING.

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future investigators to duplicate previous work as closely as possible.

Various techniques do exist that allow investigators to manipulate or transform data so that it may be possible to compare them with data collected in other ways. Nevertheless, we recommend that investigators use standard protocols whenever possible. Our ignorance of reptile biodiversity (and the general biodiversity of the planet) is enormous, and habitat destruction and species loss are proceeding apace. Time is short, personnel are few, and resources are limited. The use of nonstandard methods to assess the biodiversity of reptiles is a luxury we can no longer afford.

Intended Audience

This volume is intended for use by government personnel, conservationists, managers, professional scientists, and amateurs throughout the world. It should be accessible to anyone with a college degree in biological or environmental science, but it should also be appropriate as a training manual in workshops for individuals with limited formal education. We view it as a self-contained volume on the basis of which a survey of reptile biodiversity can be accomplished. Nevertheless, we have provided extensive references to books and to the primary literature so that individuals working in especially difficult areas or habitats or with especially problematic data can access specialized information applicable to unusual situations. In keeping with our theme of standardization, we have attempted to adhere to the taxonomic names (scientific and standard English) found in Crother (2008, as updated 31 March 2011), ITIS (Integrated Taxonomic Information System, http://www.itis.gov/), and *The Reptile Database* (Uetz et al. 2011). Our purpose is not to endorse any particular naming system or taxonomic decision, but rather, to minimize confusion. We have also abbreviated or eliminated sections on sources of supplies, chemical formulae, and so forth, which may have been more extensive in other volumes, because such information is easily available on the increasingly ubiquitous Internet. We emphasize quantitative methods over the simple listing of species present in an area because such data are of much greater utility in recognizing and documenting changes in population levels. We recognize, however, that quantitative surveys are not always possible, particularly when time, personnel, and other resources are limited. Nevertheless, any information that contributes to our knowledge of species' distributions (both geographically and ecologically) and seasonal activities is worthwhile, and we encourage researchers to take advantage of opportunities to work in poorly known areas.

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CHAPTER TWO

Reptile Diversity and Natural History: An Overview

ROY W. McDIARMID

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Introduction

Reptiles represent one of the more successful evolutionary radiations known, and their living representatives include a diverse and distinctive set of aquatic, terrestrial, and arboreal taxa that occur nearly everywhere in the world. They encompass all scaled tetrapods with an amniotic egg that lack hair and mammary glands (mammals) and feathers (birds). While some disagreement about placement of turtles exists, for this presentation I have adopted a recently proposed classification that treats turtles, crocodilians, tuataras, lizards, snakes, and the aberrant volant reptile group called birds (not treated further) as Reptiles (Fig. 1).

The earliest fossil record of a reptile dates from the Pennsylvanian period, about 300 million years ago. During the period since then, many fossil lineages (not shown in Fig. 1) have become extinct. Those that remain (exclusive of birds) are the subject of this volume; they comprise about 9,088 living species currently placed in about 1,109 genera and distributed across five groups as follows: turtles (326 species), crocodilians (24), tuataras (2), lizards (5,519), and snakes (3,217). Among the amniotes, the diversity of the reptiles, when compared to those of the living mammals (about 5,420) and birds (about 10,500), attests to their evolutionary success. Because turtles, crocodilians, and lepidosaurs (tuataras, lizards including amphisbaenians, and snakes) are relatively distantly related, I have tried to capture the highlights of the biological diversity of each group in its own account.

Understanding something about the diversity, distribution, habitat use, and natural history of each species of reptile that is likely to occur in a particular region or at a specific site is essential for an inventory project to be successful. Therefore, I strongly recommend that anyone unfamiliar with the herpetofauna of a region to be surveyed review the literature on the ecology of that region, paying particular attention to habitats likely to be used by reptiles. To support such an effort, I have listed regional references for each of the major groups of reptiles at the end of its section. These publications will provide an introduction to the herpetofaunas and facilitate compilation of working lists of species likely to be present. Once a list has been compiled, investigators should become familiar with the ecology and general natural history of those species by reading descriptive accounts in the literature. If possible, investigators should also visit a museum with reptile collections from the targeted country and/or region (see Appendix I) to familiarize themselves with targeted species, especially those that they have not encountered previously. Such an exercise will provide an understanding of the reptile diversity of the area and also facilitate selection of suitable sampling methods and field gear to be used during the project. Such preparation also increases the effectiveness of the sampling effort and, therefore, the accuracy with which the reptile diversity and abundance can be estimated.

To accompany the narrative, I have prepared tables that briefly summarize the diversity, continental distributions, and general habitat use of the groups of living reptiles, except tuataras: turtles, Table 1; crocodilians, Table 2; lizards, Table 3; and snakes, Table 4. The diversity counts (genera and species) are current through June 2010. These data were extracted from the literature, some of which is listed by geographic area at the end of each of the five group accounts; draft manuscripts and species lists to which I had access; and colleagues with detailed knowledge of specific groups. Some of my diversity counts were checked against the summary data contained in the Reptile Database (http://www.reptile-database.org/), but all estimates are mine.

The habitat designations for each group (family or subfamily) represent my best estimates of the primary habitats used by their inclusive species and vary among tables according to their suitability for each group. Lists of species were compiled

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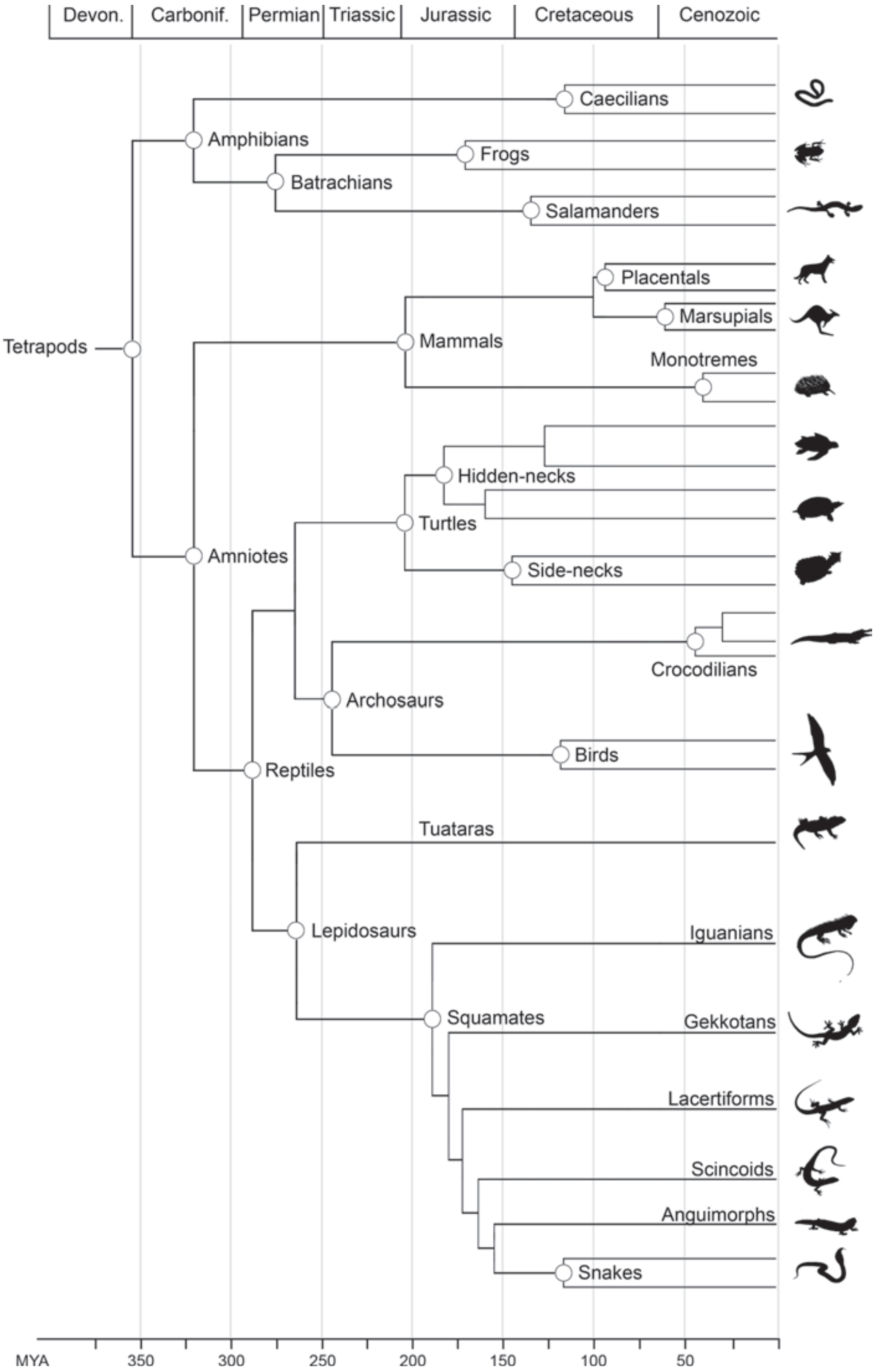

FIGURE 1 A representative chronogram showing tetrapod phylogeny and the relationships among groups of living reptiles and between reptiles and other familiar vertebrate groups. The figure is based on Hugall et al. (2007, fig. 5). The upper scale depicts geologic eras, and the lower scale is millions of years before present (MYA).

for each genus and their habitat preferences summarized by taxonomic group and continental area. Habitats are listed alphabetically in each table, with no effort to reflect frequency of their use within each group. These estimates are derived from my reading of the literature and my personal experience with certain groups in specific regions; they are conservative assignments offered to provide the field investigator with some idea of where species are likely to occur at a site. They do not represent every occurrence of a species in a habitat. For example, if a single individual of a species that is known to be arboreal was once found crossing a trail at night, I did not score it as both terrestrial and arboreal for this review. My experience with the tropidurine iguanid lizard, *Uracentron flaviceps,* illustrates the point. The only two specimens that I have seen in the field were males collected as they swam in rivers at two different sites in Amazonian Peru. If I scored these as aquatic, as the field data indicate, then future researchers might waste a lot of time looking for these lizards in rivers in the Amazon Basin; in fact, the species is an arboreal form that spends most of its time on horizontal branches high in the canopy. Presumably, these two males were either pushed (during a territorial dispute?) or jumped (to escape from a predator?) from a limb in the canopy of a tree that extended over the water.

I also discuss interesting and relevant aspects of the natural history of reptiles in each major group. These selections are not meant to be inclusive. Rather, they represent various elements of reptile biology that I find interesting, and that I think will be useful in understanding diversity, distribution, and abundance data. They should also facilitate the formulation of a study design and selection of appropriate methods for sampling different types of reptiles in different kinds of habitats.

Turtles

Turtles, a distinctive and easily recognized group of reptiles, are an important component of aquatic and terrestrial ecosystems throughout the world. Nearly all living species have a bony shell that encloses their pectoral and pelvic girdles; the exceptions are a few aquatic forms in which the shells are soft and leathery. The earliest known fossils of this group, which date from the Late Triassic more than 200 million years ago, also had bony shells and were semiaquatic. Although the general body plan of turtles is consistent throughout the taxon, extant species are morphologically diverse, with various groups typically characterized by differences in the way the neck retracts and in the size and shape of the shell. The two modern-day clades of turtles, the Pleurodira (side-necked turtles) and Cryptodira (hidden-necked turtles), are distinguishable through most of their fossil history. Members of both groups are able to withdraw their heads, limbs, and tails into their shells for protection. The pleurodires, which are aquatic, move the head and neck to the side into a space between the dorsal carapace and the ventral plastron, but these structures remain exposed from the side. In contrast, the cryptodires, which are found in terrestrial, freshwater, and marine habitats, retract the neck in a vertically oriented S-shape into a medial space within the body cavity so that only the top of the snout is visible between the shell surfaces and the retracted forearms. In a few species of cryptodires, the shell is also hinged either dorsally or ventrally, allowing its nearly complete closure and protection of the soft parts. North American box turtles (*Terrapene* spp.) are familiar examples of species with this structural modification.

Even though the Testudines is an old group of tetrapods, its diversity is relatively low for having had such a long, successful evolutionary history. Currently about 326 species in 95 genera are recognized. Most of these are tropical in distribution, with some notable temperate exceptions (e.g., the high diversity of turtles in the southeastern United States). Interestingly, turtle diversity by continent, in part reflecting their Gondwanan history, is remarkably balanced, with the highest number of species occurring in Asia (41 genera and 98 species), and other major tropical areas having between 18 and 23 genera and from 50 to 58 species (Table 1).

Male turtles have a median penis, fertilization is internal, and females are *oviparous* (i.e., they lay eggs). Males often are larger than females, particularly in larger species, but the reverse may be characteristic of smaller species, where females exceed males in carapace length. Males usually have larger and longer tails than females and, in many species, a concave plastron to facilitate mounting of the female during copulation. Males of many tortoises vocalize during copulation, often with species-specific sounds. Courtship in some aquatic species of terrapins (Emydidae) is elaborate and involves males with long claws stroking or drumming on the heads of females at the water's surface. Females of most species excavate terrestrial nests with their hind legs in a stereotypic fashion and, depending on the species and adult size, deposit between 1 and 160 eggs in an urn-shaped chamber. In marine species, females often return to natal beaches and lay up to five clutches in a single reproductive season. Hatchlings of most turtles grow slowly and take years to attain sexual maturity. However, most species are long lived and have multiple reproductive bouts in a lifetime.

Turtles are remarkably diverse ecologically and occupy terrestrial, freshwater, and marine habitats throughout the world. Turtles have been especially successful in occupying the tropical oceans of the world, and several marine species have the largest distributions of any tetrapod. The massive (carapace length to 1.6 m, body mass to 950 kg) Leatherback Sea Turtle (*Dermochelys coriacea*) occurs in all the marine environments, even the cool waters of the north- and south-temperate oceans, an ability facilitated by its unique system of inertial endothermy. Its fusiform shape, long and highly modified front flippers, lack of epidermal scutes on the shell (which consists instead of thousands of small bones covered by a thin smooth skin), and carapace with seven longitudinal keels are adaptations for swimming. It is truly pelagic, and its migratory patterns have been well documented with satellite telemetry and depth gauges. It feeds almost exclusively on jellyfish. The age at sexual maturity is not known, and copulation has rarely been observed.

Most turtles, whether aquatic or terrestrial, have hard shells. In the terrestrial testudinids the hind feet are elephantine, and the shells solid and sometimes highly domed; the scutes often show annual growth rings, and individuals may be long lived (up to 100 y). Some species dig deep burrows. Most tortoises are herbivores, but many attain large sizes. Some of the giant tortoises found on Aldabra and the Galapagos Islands may reach weights of 300 kg (>660 lbs) in the wild. Unlike Aldabra Tortoises (*Aldabrachelys gigantea*), Galapagos Tortoises (*Chelonoidis* spp.) are sexually dimorphic, with males often 2 to 3 times the size of females. Shells of some Galapagos Tortoises (e.g., *C. duncanensis* and *C. hoodensis*) are strongly saddle-backed.

The shells of some freshwater turtle species (e.g., Trionychidae) are round, flattened, and generally smooth, without epidermal scutes; they have reduced bony components and lack plastral buttresses. Collectively, these species are called softshell turtles. They usually have long necks and tube-shaped snouts that enable a submerged individual to breathe with minimal exposure. Some species are fast-swimming carnivores that actively forage for invertebrates and other organisms; others seek cover in bottom debris or bury themselves in sand and wait for unsuspecting prey to pass. Some get quite large, with shells more than 1 m long and weights up to 120 kg.

As a group, turtles are one of the most exploited organisms in the world. Sea turtles and their eggs have been harvested as food for hundreds of years, and giant tortoises were often captured and stored on board ancient ships as food for sailing crews. Turtles are commonly eaten throughout the world, but especially in China and in countries of the Pacific Islands, and the Caribbean. Several species have been exterminated by humans in their quest for food, medicine, jewelry,

TABLE 1

Diversity,[a] Geographic Distribution,[b] and Primary Habitats[c] of Turtle Families (Testudines)

Taxon	*Diversity*	*North America*	*Middle America*	*South America*	*Europe*	*Africa*	*Asia*	*Australia*
TESTUDINES	95 (326)	22 (56)	19 (55)	19 (58)	6 (10)	23 (57)	41 (98)	18 (50)
Pleurodira								
Chelidae	14 (61) aq	0	0	7 (23) aq	0	0	0	7 (38) aq
Pelomedusidae	2 (19) aq	0	0	0	0	2 (19) aq	1 (1) aq	0
Podocnemididae	3 (8) aq	0	0	2 (7) aq	0	1 (1) aq	0	0
Cryptodira								
Carettochelyidae	1 (1) aq	0	0	0	0	0	0	1 (1) aq
Chelonidae	5 (6) mr	4 (4) mr	4 (4) mr	4 (4) mr	2 (2) mr	4 (4) mr	4 (4) mr	5 (5) mr
Chelydridae	2 (4) aq	2 (2) aq	1 (2) aq	1 (1) aq	0	0	0	0
Dermatemydidae	1 (1) aq	0	1 (1) aq	0	0	0	0	0
Dermochelyidae	1 (1) mr	1 (1) mr	1 (1) mr	1 (1) mr	1 (1) mr	1 (1) mr	1 (1) mr	1 (1) mr
Emydidae	12 (48) aq, sa, tr	11 (33) aq, sa, tr	5 (19) aq, sa, tr	1 (3) aq	1 (2) aq	1 (1) aq	1 (1) aq	0
Geoemydidae	19 (70) aq, sa, tr	0	1 (5) aq, sa, tr	1 (4) aq, tr	1 (2) aq	1 (1) aq	17 (59) aq, sa, tr	2 (2) aq
Kinosternidae	4 (25) aq	2 (10) aq	3 (18) aq	1 (3) aq	0	0	0	0
Platysternidae	1 (1) aq	0	0	0	0	0	1 (1) aq	0
Testudinidae	17 (51) tr	1 (3) tr	2 (4) tr	1 (12) tr	1 (3) tr	10 (25) tr	5 (10) tr	1 (1) tr
Trionychidae	13 (30) aq	1 (3) aq	1 (1) aq	0	0	3 (5) aq	10 (21) aq	1 (2) aq

a. Numbers of living genera and species (in parentheses) in each family, and total for Testudines.

b. Presence of species by continent: North America = United States and Canada; Middle America = Mexico, Central America, and the Caribbean; South America includes Trinidad; Europe = area west of 36° E (Arnold 2002); Africa includes Madagascar and the Seychelles; Asia = area east of 36° E and west of Wallace's Line; Australia = area east of Wallace's Line, including New Guinea, New Zealand, and some Pacific Island groups.

c. Characterization of turtle species in each family by the primary habitats used: aquatic (aq) = in freshwater rivers, streams, lakes, ponds, marshes, and swamps; semiaquatic (sa) = live (and feed) at interface between aquatic and terrestrial habitats; terrestrial (tr) = in forests, savannas, deserts, and other terrestrial habitats; marine (mr) = in warmer waters of the world's oceans.

and aphrodisiacs. The primary threats to turtle populations today include exploitation for food and commercial products (e.g., tortoise shell), loss of habitat in the face of expanding human populations and urban development, and the pet trade. The food market in China has had and is having a profound effect on the conservation of turtle populations in Asia and other parts of the globe. Major efforts to protect the nesting beaches of marine species have benefited some populations, but much remains to be done.

Some informative general references on turtles include Bonin et al. (2006), Ernst and Barbour (1989), Iverson (1992), King and Burke (1989), Obst (1988), Orenstein (2001), Rhodin et al. (2008), Vitt and Caldwell (2009), Wermuth and Mertens (1961), and Wyneken et al. (2008). In addition, many useful regional treatments include turtles: for North America, see Buhlmann et al. (2008), Carr (1952), Ernst and Lovich (2009), Vetter (2004b); for Middle America, see Crother (1999), Köhler (2003), Powell and Henderson (1996), Savage (2002), Schwartz and Henderson (1991), Vetter (2005), Wilson et al. (2010); for South America, see Carreira et al. (2005), Cei (1993), Duellman (2005), Pritchard and Trebbau (1984), Rueda-Almonacid et al. (2007), Vetter (2005); for Europe, see Arnold (2002), Sindaco and Jeremčenko (2008), Vetter (2004a); for Africa, see Boycott and Bourquin (2000), Branch (1998), Glaw and Vences (2007), Kuchling and Garcia (2003), Pedrono and Smith (2003), Spawls et al. (2002), Vetter (2004a); for Asia, see Chan-ard et al. (1999), Cox et al. (2010), Das (2010), Nguyen et al. (2009), Nutaphand (1979), Sindaco and Jeremčenko (2008), Tikader and Sharma (1985), Vetter and van Dijk (2006), Ziegler (2002); and for Australia, see Cann (1998), Goode (1967), and Wilson and Swan (2003).

Crocodilians

There are 24 species of living crocodiles, which are distributed widely in tropical regions of the world. Like turtles, crocodilians have a long fossil history, dating from the Late Triassic, more than 220 million years ago. The fossils are abundant, and many exhibit modifications of the basic crocodilian body plan of a semiaquatic predator with a large head, elongate snout, strongly toothed jaws, and dorsal sculpturing of the skull with ridges and pits; a diaphragm and a four-chambered heart; a thick robust body with short but well-developed legs with clawed, webbed feet, and a long, thick, laterally compressed tail; and bony dorsal body armor of distinct osteoderms covering the neck, body, and tail. Some early forms were terrestrial, relatively small, long-legged lizard-like forms with relatively short snouts. Two major evolutionary trends were repeated multiple times through their long history. One was associated with an aquatic, probably marine, existence and marked by snout elongation, paddle-like limbs, and fish-eating habits. The other was associated primarily with a terrestrial existence and featured a broadening and deepening of the snout, serrated teeth, and more carnivorous habits. These general patterns were accompanied by morphological shifts that involved the development of a secondary bony palate, a shift of the internal nostrils to more posterior positions, a more flexible spinal column, modifications of the ankle joint, and several other notable traits that have contributed to the success of this widely distributed and important semiaquatic freshwater carnivore that we know today as living crocodiles.

All modern crocodiles have internal fertilization, and males have a median penis. All species are oviparous and have a complex social structure. Males and females are territorial, and both sexes are highly vocal, especially during courtship and breeding. Females usually construct terrestrial nests of decaying vegetation or dig a nest cavity in sand. Nesting is annual and seasonal, and its timing varies among species and habitats. Clutches include from 10 to about 150 hard-shelled eggs, the number usually increasing with female body size both among and within species. The sex of hatchlings is determined by the incubation temperature. Nest attendance, usually by the female, is common, and parental care, including opening nests, breaking egg shells and freeing young, and transporting hatchlings to water, has been well documented in several species. Vocal communication between hatching young and the attending parent is also well known and may facilitate guarding of the crèche by the parent.

Living crocodilians are classified into three families (Table 2). The Alligatoridae includes eight species in four genera; all but one are restricted to freshwater habitats in the Americas. The two species of alligators are subtropical in distribution. The Chinese Alligator (*Alligator sinensis*) is restricted to the lower Yangtze River in China and reaches 2 to 3 m in total length. The much larger American Alligator (*Alligator mississippiensis*), in contrast, may grow to 5 m and weigh more than 500 kg. The species is widespread in freshwater marshes and other wetlands (occasionally entering brackish habitats) in the southeastern United States. These two subtropical species make frequent use of burrows, especially during cooler months. Like other species of crocodilians, alligators are carnivorous. Smaller individuals eat snails, clams, and insects and then, as they grow, gradually shift to larger prey, including rats, snakes, turtles, slow-moving fish, and small mammals and birds. Large adults sometimes prey on large mammals, such as dogs, deer, calves, and occasionally people.

The other alligatorids include the Black Caiman (*Melanosuchus niger*), which is the largest predator in South America, reaching more than 6 m in length. The species is found in oxbow lakes and slower-moving parts of rivers in Amazonian and Guayanan South America. Young feed on mollusks and other invertebrates, gradually shifting to fish, reptiles, small mammals, and birds as they grow; large individuals have been reported to eat other caiman. The three species of *Caiman* (*C. crocodilus*, Common Caiman; *C. latirostris,* Broad-snouted Caiman; *C. yacare,* Yacare Caiman) are smaller (2–3 m long) and occur in a variety of freshwater habitats including rivers, lakes, coastal streams, and swamps from southwestern Mexico through Central America and most of tropical South America. Young caimans eat insects, crabs, and other invertebrates and gradually change to large aquatic snails, fish, and small mammals and birds, as they grow. The two species in the genus *Paleosuchus* (*P. palpebrosus,* Cuvier's Dwarf Caiman; *P. latirostris,* Schneider's Dwarf Caiman) are among the smallest crocodiles (1–1.7 m total length); they are heavily armored and more terrestrial than other forms and often spend time in burrows or beneath logs. Dwarf-caiman species are sometimes called smooth-fronted caimans because they lack a bony ridge in front of the orbits. They occur in much of South America in the Orinoco and Amazon basins, in Guyana, Suriname, and French Guiana, and in the upper reaches of the Paraguay and Paraná River systems, usually along small forest streams and in areas of flooded forest. Hatchlings and juveniles eat many kinds of invertebrates (terrestrial insects, crustaceans) and small vertebrates (fish and tadpoles); they gradually shift to more-terrestrial vertebrates (frogs, snakes, lizards, birds, and small mammals) as they grow. Adults occasionally eat larger mammals and other crocodilians. Usually, only a single species of caiman occurs in suitable habitat in South America; however, in some parts of the Amazon Basin as many as three species may co-occur. In the lower reaches of the Río Tambopata in southeastern Peru, for example, I have observed or collected Black, Common, and Dwarf caiman. Typically, *Melanosuchus niger* is found in large ox-bow lakes (e.g., Lago Tres Chimbadas) in the region, while *Caiman crocodilus* frequents the edges of the river and mouths of quebradas. *Paleosuchus trigonatus* occurs in small forest streams and temporary forest ponds.

The Crocodylidae includes three genera: *Crocodylus* with 12 species, and *Mecistops* and *Osteolaemus* with one each. They are among the largest freshwater and coastal marine predators known; some individuals of *Crocodylus porosus* (Estuarine Crocodile) reach a length of more than 7 m and a mass of more than 1,000 kg. Although most species live in freshwater, several venture into brackish water, and one is considered a saltwater species. Interestingly, one brackish water or marine species occurs in most continental areas (Table 2). Although six freshwater species of crocodiles dig nest holes in sandy areas, others (e.g., *C. mindorensis, C. moreletii, C. siamensis*) build mound nests, and two (*C. acutus* and *C. rhombifer,* American and Cuban crocodiles, respectively) either dig holes or build mounds, depending on the habitat and circumstance. The *Mecistops* and *Osteolaemus* species are African freshwater forms that build mound nests. As with most crocodilians, the diet of crocodiles varies with age. Hatchlings and young eat small prey, including insects, spiders, frogs, small snakes, and other small vertebrates. Marine and brackish forms seem to eat more crabs, shrimp, and fish. As crocodiles grow, they add larger prey (small mammals, birds, turtles) to the diet, and adults of large species will take large birds and mammals (warthogs, zebra, antelope, buffalo, and humans). With few exceptions, most species are opportunistic and will grab and eat whatever prey of suitable size is available.

The Gavialidae includes two living species, in separate genera. The Gharial (*Gavialis gangeticus*) occurs in several large rivers of the Indian subcontinent. It is a large animal (up to 7 m total length) and the most aquatic of living crocodiles.

TABLE 2

Diversity,[a] Geographic Distribution,[b] and Primary Habitats[c] of Crocodilian Families (Crocodylia)

Taxon	*Diversity*	*North America*	*Middle America*	*South America*	*Europe*	*Africa*	*Asia*	*Australia*
CROCODYLIA	9 (24)	2 (2)	3 (5)	4 (8)	0	3 (3)	4 (8)	1 (3)
Alligatoridae	4 (8) aq	1 (1) aq	2 (2) aq	3 (6) aq	0	0	1 (1) aq	0
Crocodylidae	3 (14) aq, mr	1 (1) aq, mr	1 (3) aq, mr	1 (2) aq, mr	0	3 (3) aq	1 (5) aq, mr	1 (3) aq, mr
Gavialidae	2 (2) aq	0	0	0	0	0	2 (2) aq	0

a. Numbers of living genera and species (in parentheses) in each family, and total for the Crocodylia.

b. Presence of species by continent: North America = United States and Canada; Middle America = Mexico, Central America, and the Caribbean; South America includes Trinidad; Europe = area west of 36° E (Arnold 2002); Africa includes Madagascar and the Seychelles; Asia = area east of 36° E and west of Wallace's Line; Australia = area east of Wallace's Line, including New Guinea, New Zealand, and some Pacific Island groups.

c. Characterization of crocodilian species in each family by the primary habitats they use: aquatic (aq) = in freshwater rivers, streams, lakes, reservoirs, marshes, and swamps; marine (mr) = in marine and brackish-estuarine waters in tropical regions.

Gharials have narrow, elongate snouts and feed primarily on fish. They also dig nest holes in sand at the edge of rivers. The False Gharial (*Tomistoma schlegelii*) is also a long-snouted, fish-eating species found in swamps, rivers, and lakes of the Malay Peninsula and larger islands of the Sunda Shelf. It reaches about 4 m total length.

Most large crocodilians with commercially valuable hides (e.g., *Alligator mississippiensis, Melanosuchus niger, Crocodylus acutus, C. intermedius, C. niloticus, C. porosus, Gavialis gangeticus, Tomistoma schlegelii*) suffered significant population losses from commercial hunting during the latter part of the 19th and first three-quarters of the 20th centuries, especially between 1930 and 1960. Today, a few species (e.g., *Crocodylus palustris, Tomistoma schlegelii*) are rarely encountered and seemingly have been extirpated from much of their former range; the Siamese Crocodile (*Crocodylus siamensis*) may be extinct in the wild. When the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) was signed in 1973, all species of crocodilians were included either on Appendix I, which lists species threatened with extinction and prohibits international commercial trade in those species, or Appendix II, which lists species that are not currently endangered but that may become so if trade is not controlled (CITES Secretariat 2010). CITES called for better record keeping and more scientifically rigorous data on crocodilian populations, and assignment of species to the two appendices was corrected and stabilized. As a result, the volume of skins traded illegally on the international market declined significantly. This reduction led to an increase in the value of legal hides. Consequently, crocodile farms for captive breeding have become a familiar component of conservation programs in many countries. This interest in crocodiles has also resulted in more effective programs for their management around the world as well as protection for critical habitat in some areas. Nevertheless, habitat loss and other types of human disturbance continue to be major problems, causing significant declines in populations of many species, including *Gavialis gangeticus*. Fortunately, conservation and management efforts directed toward restocking in areas from which species have been extirpated have had some positive results.

The Crocodilians web site (http://crocodilian.com/) is a high-quality source of information on the biology of crocodilians. Other useful general references for this group include Grenard (1991), Grigg et al. (2001), Groombridge (1987), King and Burke (1989), Neill (1971), Ross (1989), Thorbjarnarson (1992), Vitt and Caldwell (2009), Webb, Manolis, and Whitehead (1987), and Wermuth and Mertens (1961). Additionally, there are many useful regional references: for North America, see Abercrombie (1989), Gibbons et al. (2009), Lang (1976), McIlhenny (1935), Thorbjarnarson (1989); for Middle America, see Crother (1999), Escobedo Galván et al. (2010), Köhler (2003), Powell and Henderson (1996), Savage (2002), Schwartz and Henderson (1991), Thorbjarnarson (1989); for South America, see Ayarzagüena (1983), Carreira et al. (2005), Cei (1993), Duellman (2005), Gorzula and Seijas (1989), Magnusson (1989), Medem (1981, 1983), Rueda-Almonacid et al. (2007), Thorbjarnarson (1989); for Africa, see Baha El Din (2006), Branch (1998), Cott (1961), Sindaco and Jeremčenko (2008); Spawls et al. (2002), Waitkuwait (1989); for Asia, see Chan-ard et al. (1999), Cox et al. (2010), Das (2010), Nguyen et al. (2009), Sindaco and Jeremčenko (2008), Whitaker (1987), Whitaker and Whitaker (1989a, 1989b); and for Australia, see Messel and Vorlicek (1989a, 1989b, 1989c), Webb and Manolis (1989), Wilson and Swan (2003).

Lepidosaurs

Tuataras

Tuataras are living remnants of a large, diverse group of reptiles that evolved in the Late Triassic and persists today only on a few small islands off New Zealand. The single extant genus, *Sphenodon,* includes two living species, *S. punctatus,* which occurs on about 30 islands off the northeast coast of North Island, and *S. guntheri,* which is restricted to North Brother Island in the Cook Strait. These are moderate-size, sexually dimorphic (males to about 60 cm total length and 1 kg body weight [$\bar{X}$ = 56 cm and 0. 66 kg]; females to about 45 cm and 0.5 kg [$\bar{X}$ = 45 cm and 0.35 kg]), lizard-like reptiles with large heads, a beak-like upper jaw, stout bodies with series of dorsal spines, and a moderately long tail (about ½ total length and often lost and regenerated); they lack a tympanum and an intromittent organ. Tuataras mate from January to March (midsummer to early fall), and females store sperm until the following spring or early summer (October to December) when they leave their home territories to nest in groups, or rookeries. An average female reproduces every 4 to 5 years; she deposits a clutch of from 5 to 18 eggs in a shallow depression or burrow, which is covered with soil and abandoned. In natural environments the incubation period is extremely long (12–16 months); in captivity incubation is shorter, suggesting that development is arrested in natural populations during colder, winter months. Hatchlings are about 100 mm long and weigh about 5 g. They have a prominent parietal eye, a striking silvery white triangular mark on the head, and are active and begin searching for food immediately following yolk absorption. Young tuataras dig short burrows or seek shelter under rocks or logs. Tuataras are slow growing; females reach maturity at about 180 mm SVL and males at about 200 mm, in 11 to 13 years. They also are long lived and reach full size only after 25 to 35 years. Some adults live more than 60 years in the wild, and one captive animal lived for more than 70 years. Researchers speculate that tuataras may reach 100 years or more. Recruitment in some areas is very low, and many populations consist almost entirely of old adults.

Adult tuataras are nocturnal and spend many of the daylight hours in burrows. On sunny days, they are often seen basking at burrow entrances. While most of the burrows are constructed as nesting sites by small petrels (Fairy Prions, *Pachyptila turtur*) and other sea birds, some are actually dug by tuataras. These burrows may be simple (one or two entrances with a single nesting chamber) or complex (several entrances leading to multiple nesting chambers). Tuataras are territorial, and even though an individual may use up to six different burrows, most burrows have only a single occupant at any one time. Single burrows may be used by several different animals, but usually at different times. The nesting sea birds tend to keep the forest floor open and free of small plants, through their frequent arrivals and departures. Their guano provides a rich mineral soil that attracts insects (e.g., beetles, crickets, spiders, moths) to areas around the burrows. Tuataras feed primarily on insects (mainly beetles) and other invertebrates but also will eat nocturnal geckos, small skinks, and the eggs and chicks of the Fairy Prions. Habitat modifications, the presence of too many large nesting petrels and shearwaters, and predation (e.g., by Norway and Polynesian rats, *Rattus norvegicus* and *R. exulans,* respectively), especially on eggs and young, are the primary threats to their continued existence.

Useful references on the biology of the species of *Sphenodon* include Newman (1982, 1987), Robb (1977, 1986), and Sharell (1966).

Squamata: Lizards

Lizards, treated here as exclusive of snakes, are the largest group of living reptiles and have successfully occupied all continents, except Antarctica, from sea level to high above the tree line. Some species of lizards are exceptionally adept at dispersing to new areas and occur on the remotest of tropical and subtropical oceanic islands. Their extraordinary evolutionary success is attributable to a complex of morphological, physiological, ecological, and behavioral traits honed by more than 100 million years of natural selection. The diversity of squamate lineages present in the Cretaceous parallels that of their Jurassic ancestors, and most of today's major lineages were present in the mid- to late Cretaceous (Fig. 1). By my count (Table 3), 5,519 recognizable species of lizards distributed across 498 genera and 34 family/subfamily lineages have been described. The species diversity of lizards is highest in the tropical and subtropical regions of Africa and Asia, followed closely by Australia, and lowest in the cool temperate, continental regions of Europe and North America.

Lizards exhibit a variety of lifestyles, many of which are representative of familial lineages; several of these lifestyles have evolved in parallel on different continents. For example, a sizeable proportion of lizard species (Agamids, Phrynosomatines, Scincids, Teiids, and Varanids) are diurnal and terrestrial, forage actively, and are common in semi-arid and arid regions of the globe (e.g., Australia, southwestern United States and northern Mexico, north Africa). The convergences in their color patterns and behaviors are remarkable.

The basic lizard body plan, which includes an elevated head, short neck, rounded trunk, four well developed limbs, and a long tail, distinguishes lizards from most other reptiles. However, limb reduction and concomitant body elongation have evolved independently in many groups, including all snakes (see "Squamate: Snakes," below). Lizards in some lineages have completely lost their limbs and successfully occupy the below-ground world as fossorial species; these lizards are moderately diverse and complementarily distributed in tropical and subtropical regions of the world (Table 3). They include worm lizards (e.g., Amphisbaenidae, Blanidae, Rhinuridae, Trogonophidae); some flap-footed geckos (Pygopodidae); acontine, felinine, and other limbless skinks (Scincidae); and blind skinks (Dibamidae). Other terrestrial forms (e.g., many gymnophthalmid and some scincid, cordylid, and anguid lizards) that occupy burrows, live in leaf litter on the forest floor, or take refuge under surface debris (rocks, fallen trees) also show varying degrees of limb reduction or loss. Finally, species in a few other terrestrial families (e.g., certain lineages of pygopodids and cordylids) are elongate, have reduced limbs, and live above ground in tussock and bunch grass; these are often referred to as grass lizards or grass swimmers.

The tropical forests of the world provide a complex arboreal environment that also has been successfully occupied by several major groups of lizards, among which the Agamidae, Chamaeleonidae, and polychrotine Iguanidae are noteworthy. Among the arboreal species are lizards that live primarily on small shrubs and trees in the forest understory, some that are active almost exclusively on the large limbs and trunks of forest trees, and a few that are known only from brightly lit forest edges or from the forest canopy. Trees with large, exposed trunks are a major component of these forests, and the dipterocarp rain forests of Southeast Asia are especially striking in this respect. Not only are they the tallest rain forests in the world, but they also have an uneven canopy with many emergent trees and many fewer vines and lianas than other comparable forests. Perhaps it is not surprising, therefore, that the diversity of gliding/parachuting vertebrates (e.g., some agamid lizards, geckos, snakes, frogs, squirrels, flying lemurs) is greater here than in forests elsewhere. Many Southeast Asian agamids have cryptic behavior and background-matching coloration, and the trunk-dwelling forms are nearly undetectable on these vertical, exposed surfaces. Yet, the open nature of the forest understory provides an opportunity for long-distance visual communication, and territorial males often advertise their presence by elaborate displays that are magnified by bright colors on hidden morphological structures. The gliding species of *Draco,* with their unique and sexually dimorphic wing-like expanses of skin (patagium) that stretch between extended ribs on either side as they parachute from one tree trunk to another and the brightly colored, extendable dewlaps

TABLE 3

Diversity,[a] Geographic Distribution,[b] and Primary Habitats[c] of Lizard Families (Sauria)

Taxon	*Diversity*	*North America*	*Middle America*	*South America*	*Europe*	*Africa*	*Asia*	*Australia*
SAURIA	498 (5519)	27 (121)	67 (856)	99 (978)	28 (71)	149 (1252)	145 (1211)	122 (1147)
Iguania								
Iguanidae								
Corytophaninae	3 (9) ar, sa, tr	0	3 (9) ar, sa, tr	1 (2) ar, sa, tr	0	0	0	0
Crotaphytinae	2 (12) sx, tr	2 (8) sx, tr	2 (10) sx, tr	0	0	0	0	0
Hoplocercinae	3 (12) ar, bu, sd, tr	0	2 (2) ar, bu, tr	3 (12) ar, bu, sd, tr	0	0	0	0
Iguaninae	8 (40) ar, bu, mr, sa, sx, tr	2 (2) sx, tr	5 (34) ar, bu, sx, tr	3 (4) ar, mr, sa, sx, tr	0	0	0	1 (3) ar
Oplurinae	2 (7) ar, bu, sx, tr	0	0	0	0	2 (7) ar, bu, sx, tr	0	0
Phrynosomatinae	9 (137) ar, bu, sx, tr	9 (47) ar, bu, sx, tr	9 (131) ar, bu, sx, tr	0	0	0	0	0
Polychrotinae	8 (407) ar, sa, sx, tr	1 (1) ar	2 (285) ar, sa, tr	8 (141) ar, sa, sx, tr	0	0	0	0
Tropidurinae	12 (365) ar, bu, sd, sx, tr	0	1 (28) bu, sd, sx, tr	11 (337) ar, bu, sd, sx, tr	0	0	0	0
Agamidae	55 (423) ar, bu, sa, sx, tr	0	0	0	1 (1) tr, sx	6 (62) ar, bu, sx, tr	38 (266) ar, bu, sx, tr	17 (94) ar, sa, sx, tr
Chamaeleonidae	9 (181) ar, tr	0	0	0	1 (2) ar	9 (177) ar, tr	1 (4) ar	0
Gekkota								
Diplodactylidae	14 (116) ar, bu, sd, sx, tr	0	0	0	0	0	0	14 (116) ar, bu, sd, sx, tr
Carphodactylidae	5 (28) ar, bu, sx, tr	0	0	0	0	0	0	5 (28) ar, bu, sx, tr
Pygopodidae	7 (40) ar, fs, sd, tr	0	0	0	0	0	0	7 (40) ar, fs, sd, tr
Eublepharidae	6 (30) ar, bu, sd, sx, tr	1 (4) sd, tr	1 (7) sd, tr	0	0	2 (4) bu, tr	3 (19) ar, sx, tr	0
Sphaerodactylidae	11 (197) ar, bu, sd, sx, tr	1 (1) sd	4 (110) ar, sd, tr	5 (52) sd	1 (1) sx	3 (19) ar, bu, sx, tr	2 (20) ar, bu, tr	0

TABLE 3 (*continued*)

Taxon	*Diversity*	*North America*	*Middle America*	*South America*	*Europe*	*Africa*	*Asia*	*Australia*
Gekkonidae	52 (837) ar, bu, sd, sx, tr	0	0	2 (4) ar, sd	2 (2) ar, sd, sx	31 (341) ar, bu, sd, sx, tr	26 (376) ar, bu, sd, sx, tr	10 (103) ar, bu, sd, sx, tr
Phyllodactylidae	11 (111) ar, bu, sd, sx, tr	1 (1) sx	3 (30) ar, sd, sx	7 (46) ar, bu, sd, sx, tr	1 (1) sd, sx, tr	3 (24) ar, sx	2 (13) sx	0
Lacertiformes								
Gymnophthalmidae	44 (217) ar, fs, sa, sd, tr	0	8 (17) ar, sa, sd, tr	44 (213) ar, fs, sa, sd, tr	0	0	0	0
Teiidae	10 (123) ar, bu, sa, tr	1 (22) bu, tr	4 (57) bu, tr	9 (59) ar, bu, sa, tr	0	0	0	0
Lacertidae	41 (299) ar, bu, sd, sx, tr	0	0	0	16 (53) ar, bu, sd, sx, tr	24 (122) ar, bu, sd, sx, tr	17 (142) ar, bu, sx, tr	0
Rhineuridae	1 (1) fs	1 (1) fs	0	0	0	0	0	0
Blanidae	1 (4) fs	0	0	0	1 (2) fs	1 (2) fs	0	0
Bipedidae	1 (3) fs	0	1 (3) fs	0	0	0	0	0
Trogonophidae	4 (6) fs	0	0	0	0	3 (4) fs	2 (2) fs	0
Amphisbaenidae	11 (152) fs	0	1 (15) fs	2 (76) fs	0	9 (62) fs	0	0
Scincoidea								
Scincidae	137 (1426) ar, bu, fs, sa, sd, sx, tr	2 (16) fs, sd, tr	5 (34) sd, tr	2 (24) tr	3 (6) fs, sd, tr	46 (333) bu, fs, sa, sd, tr	48 (329) ar, bu, fs, sa, sd, tr	66 (707) ar, bu, fs, sa, sd, sx, tr
Cordylidae	8 (89) ar, bu, fs, sx, tr	0	0	0	0	8 (89) ar, bu, fs, sx, tr	0	0
Xantusiidae	3 (29) sd, sx, tr	1 (6) sx, tr	3 (26) sd, sx, tr	0	0	0	0	0
Anguimorpha								
Anguidae	13 (117) ar, bu, sd, tr	4 (11) ar, sd, tr	10 (49) ar, sd, tr	2 (8) tr	2 (3) sd, tr	1 (1) sd, tr	2 (6) bu, sd, tr	0
Helodermatidae	1 (2) bu, tr	1 (1) bu, tr	1 (2) bu, tr	0	0	0	0	0
Varanidae	2 (70) ar, bu, sa, sx, tr	0	0	0	0	1 (5) ar, bu, sa, sx, tr	2 (14) ar, bu, sa, tr	1 (54) ar, bu, sa, sx, tr
Shinasauridae	1 (1)	0	0	0	0	0	1 (1)	0

(*continued*)

TABLE 3 (*continued*)

Taxon	*Diversity*	*North America*	*Middle America*	*South America*	*Europe*	*Africa*	*Asia*	*Australia*
	sa						sa	
Xenosauridae	1 (6) sx	0	1 (6) sx	0	0	0	0	0
incertae sedis								
Dibamidae	2 (22) fs	0	1 (1) fs	0	0	0	1 (19) fs	1 (2) fs

a. Numbers of living genera and species (in parentheses) in each family, and total for the Sauria.

b. Presence of species by continent: North America = United States and Canada; Middle America = Mexico, Central America, and the Caribbean; South America includes Trinidad; Europe = area west of 36° E (Arnold 2002); Africa includes Madagascar and the Seychelles; Asia = area east of 36° E and west of Wallace's Line; Australia = area east of Wallace's Line, including New Guinea, New Zealand, and some Pacific Island groups.

c. Characterization of lizard species in each family by the primary habitats used: aquatic (aq) = in freshwater rivers, streams, lakes, ponds, marshes, and swamps; semiaquatic (sa) = at interface between aquatic and terrestrial habitats; terrestrial (tr) = on ground in forests, savannas, deserts, and other terrestrial habitats; burrow dweller (bu) = occupy burrows they excavate or those excavated by other organisms; fossorial (fs) = burrowed beneath the ground surface in sand and loose soil; surface debris (sd) = in forest leaf litter, or beneath logs, rocks, and other surface debris; arboreal (ar) = above ground on bushes, tree trunks, or in tree canopy; saxicolous (sx) = found on or among rocks; marine (mr) = in warmer waters of the world's oceans.

that they display in grand rituals under the forest canopy, likely epitomize these trends toward both crypsis and highly visual displays. In addition to the striking sexual dimorphism in body morphology and coloration of many arboreal lizards, other morphological traits also exemplify this existence. For example, some species of the primarily arboreal African family Chamaeleonidae have striking morphological traits that include a laterally compressed, leaf-like body, an ability to change color quickly, a prehensile tail, turret-like and independently movable eyes, a projectile tongue, and *zygodactyl* digits (two pointing forward and two backward), all of which adapt them for arboreality. In addition, however, they sometimes have ornate spines, horns, rostral protuberances, flaps, crests, and other structures that they use in displays and combat. Equally amazing morphologies are known in other arboreal lineages within the Southeast Asian agamids (e.g., *Acanthosaura, Gonocephalus,* and *Lyriocephalus*) and some New World hoplocercine (e.g., *Enyalioides*) and polychrotine (e.g., *Chamaeleolis*) iguanids.

Many other species of lizards are *saxicolous,* living in and on rocky substrates (see "Rock-Dwelling Reptiles," in Chapter 11). Several of the iguanid lineages contain well-known species common in rocky habitats in drier regions of the New World. Agamid lizards occupy similar rocky areas in the deserts of Australia, Asia, and Africa, and they exhibit color patterns, life histories, and other traits comparable to those of the iguanids. Saxicolous species are also well represented in the Cordylidae, Varanidae, Xenosauridae, and several gekkotan groups, especially the Phyllodactylidae. The toes of many geckos are modified to form remarkable adhesive pads that facilitate climbing on vertical surfaces such as tree trunks and rock faces. Such structures also make it easy for species to ascend walls in buildings and feed on insects that are attracted to lights at night. Vocal communication is common among many nocturnal, edificarian species, some of which are known only from collections made in and around human habitations.

In contrast to snakes, only a few species of lizards are semiaquatic in freshwater habitats (e.g., Corythophaninae, Polychrotinae, Varanidae, Shinasauridae), and only one has successfully invaded the marine environment (Iguaninae).

Maintenance of a relatively high body temperature is critical for normal lizard activity, and thermoregulatory behavior is an integral part of lizard ecology. Everything related to prey capture, predator escape, and mating success, as well as physiological processes such as digestion, reproduction, and growth, are temperature dependent. Most lizards are diurnal ectotherms, although some groups are primarily nocturnal (e.g., Gekkonidae, Xantusiidae). Species with diurnal, nocturnal, and crepuscular activity patterns occur in many families and are likely to be found on most continents. Body sizes of lizards vary from small (15–20 mm SVL; e.g., in certain terrestrial sphaerodactyline gekkos) to very large (more than 3 m total length and 150 kg in the Komodo Dragon, *Varanus komodoensis*). An extinct Pleistocene varanid from Australia (*Megalania prisca*) was truly gigantic and is estimated to have weighed more than 600 kg and measured more than 6 m total length.

Most lizards eat insects and other small arthropods, but many others are herbivorous, carnivorous, or omnivorous. Some species are *sit and wait* (ambush) predators (e.g., many species of Iguania), whereas others forage actively (e.g., most gekkotans, lacertiforms, scincoids, anguids, varanids). These two foraging modes can influence both the size and type of prey eaten, as well as determine the diel and seasonal activity patterns of some species. Foraging habits are also reflected in general patterns of coloration (mottled and cryptic vs. striped and obvious). Some lizards are specialists, eating only specific kinds of insects; other species are generalists. Ant specialists are well known within the Iguania (e.g., horned lizards, *Phrynosoma* spp., in the southwestern United States and Mexico; thorny devils, *Moloch horridus,* in Australia; and some flying lizards, *Draco* spp. of Southeast Asia.), whereas termite eaters are more likely to be found among species of geckos, teiids, and skinks. Crotaphytine iguanids eat larger prey, including other lizards, as does the pygopodid *Lialis burtonis*. Larger lizards often eat larger prey, and the two species of *Heloderma* eat small mammals and birds and their eggs, which are taken whole. Varanid lizards eat small to large invertebrates, as well as vertebrates (e.g., lizards, birds and their eggs). Some large varanids (e.g., Komodo Dragons, *Varanus komodoensis*) are carnivores and can bring down a pig or deer; they also are large enough to kill and eat a human. A few other lizards are herbivorous, and some species shift their diets seasonally, showing a preference for yellow flowers of certain plants(!). Young of a few species of *Ctenosaurus* (Iguaninae) eat insects and shift to plants as adults. Marine iguanas (*Amblyrhynchus cristatus*) feed underwater on a wide variety of algae that grow on the shallow reefs of the Galápagos Islands.

Unlike turtles and crocodilians, squamates have paired copulatory organs (hemipenes). Most lizards lay their eggs in nest chambers that they dig in the soil, bury them among rotting vegetation, or place them under tree bark and cap rocks. The eggs of many geckos have calcified shells and are resistant to desiccation, whereas those of most other lizards have leathery shells and rely on a relatively moist environment for proper development. About 20 percent of all lizards are viviparous, with live-bearing species known from at least 11 families (i.e., Agamidae, Chamaeleonidae, Iguanidae, Diplodactylidae, Lacertidae, Trogonophidae, Scincidae, Cordylidae, Xantusidae, Anguidae, and Xenosauridae). Phylogenetic evidence indicates that live birth has evolved multiple times within lizards, occasionally even within a single family (e.g., Scincidae). Within these families, viviparity is more likely to occur in species that live in cool, temperate areas or at high elevations in the tropics. Clutch size varies from 1 to more than 60 eggs. So-called fixed (genetically determined) clutch size is characteristic of several lizard groups (e.g., all species of *Anolis* and sphaerodactylid geckos lay single eggs, but they produce clutches in rapid succession, whereas many gecko species lay 2 eggs, 1 per ovary). Communal nesting is common among many species of geckos whose eggs adhere to hard surfaces. The lizards frequently deposit these peculiar eggs under the bark of trees, which may account for the wide distributions of certain species of geckos on oceanic islands. Although the majority of lizards reproduce sexually, *parthenogenesis* (i.e., asexual reproduction without males) occurs in more than 30 species in seven or eight families. It arises through hybridization between two sexually reproducing species. Some all-female, parthenogenetic species are diploid, others are tetraploid, and many live in ecotonal habitats.

Reproduction is seasonal in many lizards, but not always in the ways we expect. Cold temperate conditions restrict reproductive activity to warmer months, and extremely dry conditions in seasonal tropical areas constrain reproductive activity to wetter seasons. Interestingly, the timing of reproduction is often shared among related taxa, rather than reflecting environmental conditions; that is, closely related species at two different sites are more likely to have similar seasonal reproductive patterns than unrelated species at the same site. Post-egg-deposition parental investment is not common in lizards; females in only a few species of skinks (e.g., *Eumeces*) and some anguids (e.g., *Gerrhonotus*) brood their eggs and thereby regulate humidity in the nest and protect the eggs against certain predators.

Other morphological and physiological modifications that characterize certain groups of lizards are reflected in their social behavior. Most lizards have color vision, and their social behaviors are easily observed and usually associated with territorial defense and mating. Two basic types of sensory modalities are apparent; color vision is especially important and reflected in the bright, often sexually dimorphic coloration of the many Iguanian lizards, most of which are diurnal. In contrast, chemical communication is primary among other forms (e.g., Lacertiformes and Scincoidea), many of which are fossorial or live in the low-light environment of the forest floor, in leaf litter, or beneath surface objects. Most gekkotan lizards are nocturnal, sexually monomorphic, and rely on chemical rather than visual cues to find mates and locate prey. Not unexpectedly, some diurnal geckos are brightly colored (e.g., *Phelsuma, Gonatodes*) and sexually dimorphic. Geckos also are the most vocal of all lizards and have been extremely successful in many habitats on all continents.

Lizards have faced some of the same threats from humans as have turtles (eaten) and crocodilians (hide trade, see Chapter 16), but generally the pressure on their populations has been less intense. Habitat modification and loss, especially the conversion of natural habitats to agricultural lands (e.g., tropical forests cleared and replaced with tree plantations or burned to create pasture, and grasslands converted to agriculture) are primary problems for lizards. The expansion of resorts, luxury villas, and tourist attractions, especially along the coasts of tropical and subtropical islands where species endemism and diversity often are high, is also a problem.

Another negative consequence of the ever-expanding human population, especially on tropical islands, has been the introduction of alien species. Initially, these species consisted of goats and pigs as a food supplement and dogs for hunting and companionship. Soon after, rats found their way to these settlements and rapidly became a problem. Their presence was often followed by intentional introductions of cats and mongooses for rat control. While sometimes effective, these control species often had an even larger negative impact on the young of large lizards and snakes and on adults of smaller vertebrates. Such sequential impacts have been well documented, especially on islands (e.g., Galapagos Islands, Round Island, St. Croix). The situation with most of the large iguanid lizards (*Cyclura* spp.) in the West Indies is typical (see Iguana Specialist Group, http://www.iucn-isg.org/actionplan/ch1/introduction.php). Seven of the nine species of these large, terrestrial lizards are listed by the United States Fish and Wildlife Service as threatened or endangered under the United States Endangered Species Act of 1973; recruitment for most of these species is relatively low. Although data are often scarce, their populations appear to consist of only a few hundred adults. Other than direct loss of habitat, the presence of introduced goats, pigs, dogs, cats, and rats has had a major negative impact on several of these forms. Head-start programs and other management/conservation schemes have been initiated for a few species and, one hopes, will have a positive impact.

Some informative general references on lizards include Pianka and Vitt (2003) and Vitt and Caldwell (2009). Additionally, many regional treatments include useful information on lizards: for North America, see Gibbons et al. (2009), Jones and Lovich (2009); for Middle America, see Campbell (1998), Crother (1999), Henderson and Powell (2009), Köhler (2003), Köhler et al. (2006), Lee (1996), Liner and Casas-Andreu (2008), Powell and Henderson (1996), Savage (2002), Schwartz and Henderson (1991), Stafford and Meyer (2000), Wilson et al. (2010); for South America, see Abuys (2003), Avila-Pires (1995), Carreira et al. (2005), Cei (1993), Duellman (2005), Murphy (1997); for Europe, see Arnold (2002), Sindaco and Jeremčenko (2008); for Africa, see Bons and Geniez (1996), Branch (1998), Eidenmüller and Philippen (2008), Glaw and Vences (2007), Nečas (2004), Pauwels and Vande weghe (2008), Schönecker (2008), Spawls et al. (2002); for Asia, see Alcala (1986), Anderson (1999), Chan-ard et al. (1999), Das (2004, 2010), Disi et al. (2001), Eidenmüller and Philippen (2008), Manthey (2008, 2010), Nguyen et al. (2009), Sindaco and Jeremčenko (2008), Somaweera and Somaweera (2009), Ziegler (2002); and for Australia, see Bauer and Sadlier (2000), Eidenmüller and Philippen (2008), Wilhelm (2010), Wilson and Swan (2003).

Squamata: Snakes

I treat snakes here as a lineage separate and distinct from those of lizards, even though its evolutionary origin is clearly embedded within the "lizard" group. Whether living snakes are more closely related to varanids or some other lineage within Sauria remains to be determined, but they do represent a monophyletic lineage that likely was derived from a terrestrial ancestor (Fig. 1). Published disagreements about the number, composition, and relationships of lineages of living snakes are many, and I have read most of them. While some readers may disagree with the implied phylogenetic structure and the number of distinct entities adopted in the organization of Table 4, I made my decisions with due consideration. I also attempted to make it easier to use these data in designing inventory projects. In most instances, I tried to be conservative and only recognize entities that seemed to have general support. In this regard, for example, I followed current thinking and included sea snakes in the Elapidae, even though I and some others could argue that recognizing one or two distinct lineages of sea snakes might better reflect their diversity and ecology and make it easier for field teams to design inventory projects to sample all snakes at sites in Southeast Asia and Australia. Representative of the several excellent research papers dealing with snake diversity that I consulted are Adalsteinsson et al. (2009), Hedges et al. (2009), and Zaher et al. (2009), but these are only a few. In my opinion, we are making good progress toward understanding snake phylogeny and diversity, and with continued, standardized sampling throughout the world, that trend will continue.

Representative snakes appear in the fossil record in the early Cretaceous some 125 million years ago. Ancestors of caenophidians ("advanced" snakes of the colubrid, elapid, and viperid groups) first appear in the Eocene, and ancestors to modern genera appear in the Oligocene. As a group, snakes have been much more successful than any of the lineages of limbless lizards, whose total species diversity is less than 1 percent that of snakes. Today, snakes occur on all continents, except Antarctica, and effectively occur in all terrestrial and aquatic habitats, including the tropical marine environment. Compared to lizards, snakes are not as effective at dispersing across expanses of water, and few occur on distant oceanic islands. Even so, their success is considerable, especially in tropical regions; it correlates well with morphological adaptations for limbless locomotion in a multitude of complex habitats and for feeding on a diversity of prey. My current count indicates that there are 3,217 species of snakes in 506 genera and about 30 family groups. The highest species diversity is recorded from Asia, followed in decreasing order by the primarily tropical and subtropical continental regions of South America, Middle America, Africa, and Australia (Table 4). In contrast, snake diversities in North America and Europe, where diversity is lowest, are only 16.1 and 0.4 percent of that in Asia and 4 and 0.01 percent of the total species diversity, respectively. Interestingly, the number of genera of snakes (506) is about equal to that of lizards (498), but the species diversity of snakes (3,217) is only about 58 percent of that of lizards (5,519). Seemingly, there are more ways to be a lizard than to be a snake. This is not surprising, given that snakes were likely derived from a burrowing ancestor within lizards that subsequently abandoned the below-ground existence to diversify in a wide variety of terrestrial habitats.

Their success in different habitats is mirrored by differences in body shape and locomotor responses for above-ground movements. Many species are secretive and occur only in specific habitats (e.g., leaf litter), but others occupy more than one habitat. Certain tropical arboreal snakes are often found on the ground, and some characteristically terrestrial species (e.g., rattlesnakes) sometimes move into low arboreal habitats to rest. Several diurnal terrestrial species (e.g., *Chironius* spp.) are most often found at night coiled in an understory tree, presumably to avoid nocturnal terrestrial predators. A trained field biologist can often predict where a snake is likely to be found by its color pattern or body shape. Diurnally active, open-habitat species are often striped or unicolor and relatively thin, whereas terrestrial forest species are often blotched and cryptic, matching the leaf litter in which they live. Arboreal snakes are often green, and the active, diurnal species (e.g., *Leptophis* spp.) are usually longer and thinner than the nocturnal, ambush predators that tend to be thick bodied and to move more deliberately (e.g., *Corallus* spp.).

Several types of locomotion are found among snakes and are not always tied to a single species or a specific habitat. The most familiar type is *serpentine locomotion,* in which contractions on opposite sides of the body form waves in the trunk that move from anterior to posterior; the lateral bends that form push the ends of the ventral scales against irregularities of the substrate, and the snake moves forward. This locomotion can be very efficient, and some snakes can move up to 11 km/h over short distances. The same motion is also used by aquatic species for swimming. With *rectilinear locomotion,* a snake progresses in a straight line as a result of wave-like movements of their ventral scales. Heavy-bodied snakes, including some pythons and vipers, often move in this fashion. In *concertina locomotion,* a snake extends and anchors its head and pulls the looped parts of its body forward; this action is repeated much like the extension and contraction of its namesake musical instrument. Snakes use this mode when moving through narrow spaces or climbing vertical surfaces such as tree trunks. *Lateralinear movement,* or *sidewinding locomotion,* is an especially effective mode for traveling over unstable substrates such as sand or mud. A snake anchors part of its body on the substrate and then lifts the remaining part of the body in a lateral loop, and reanchors it. This "anchor, release, lift, and anchor" type of movement pushes the snake forward but also laterally, leaving behind the characteristic J-shaped marks found in sand dunes in many deserts of the world. I have tracked desert vipers in coastal Namibia and rattlesnakes on dunes in southern California by following these tell-tale trails. Other small snakes that live in sandy habitats often engage in *subsurface sand swimming*; countersunk jaws and valves on their nostrils keep out the sand. *Gliding,* or *parachuting,* is the final locomotory mode; it is used by some arboreal snakes (e.g., *Chrysopelea* spp.) in the forests of Southeast Asia. Following an initial launch, they flatten the body, form a concave, parachute-like surface, and swim through the air (see http://www.flyingsnake.org/).

Why snakes have been more successful than other monophyletic lineages of "lizards" characterized by limb loss has been the subject of considerable inquiry. The lack of movable eyelids and external ear openings seemingly were ancestral characteristics that contributed to their above-ground diversification. Snakes also have an elongate body, an increased number of vertebral segments (each with paired ribs) in the trunk region, and greater overall body flexibility as a result of modifications of intervertebral connections (i.e., zygantra and zygosphenes). In addition, some paired organs are elongated

TABLE 4

Diversity,[a] Geographic Distribution,[b] and Primary Habitats[c] of Snake Families (Serpentes)

Taxon	*Diversity*	*North America*	*Middle America*	*South America*	*Europe*	*Africa*	*Asia*	*Australia*
SERPENTES	506 (3217)	48 (143)	125 (677)	106 (727)	17 (35)	132 (655)	167 (862)	88 (359)
Scolecophidia								
Anomalepididae	4 (17) fs, sd	0	3 (3) fs, sd	4 (14) fs, sd	0	0	0	0
Typhlopidae	9 (242) fs, sd	0	1 (42) fs, sd	2 (8) fs, sd	1 (1) fs, sd	5 (59) fs, sd	5 (62) fs, sd	5 (66) fs, sd
Leptotyphlopidae	12 (116) fs, sd	1 (3) fs, sd	5 (21) fs, sd	4 (38) fs, sd	0	6 (57) fs, sd	1 (6) fs, sd	0
Alethinophidia								
Anomochilidae	1 (3) fs, sd	0	0	0	0	0	1 (3) fs, sd	0
Uropeltidae	8 (49) fs, sd	0	0	0	0	0	8 (49) fs, sd	0
Cylindrophiidae	1 (10) fs, sd	0	0	0	0	0	1 (4) fs, sd	1 (7) fs, sd
Aniliidae	1 (1) fs, sd	0	0	1 (1) fs, sd	0	0	0	0
Macrostomata								
Xenopeltidae	1 (2) bu, sd, tr	0	0	0	0	0	1 (2) bu, sd, tr	1 (1) sd
Loxocemidae	1 (1) fs, tr	0	1 (1) fs, tr	0	0	0	0	0
Pythonidae	9 (40) ar, bu, sa, sd, sx, tr	0	0	0	0	1 (4) bu, sa, sx, tr	2 (7) ar, sa, tr	7 (29) ar, sd, sx, tr
Boidae	12 (47) aq, ar, bu, fs, sd, tr	2 (3) sd, tr	4 (16) ar, tr	4 (13) aq, ar, tr	1 (1) bu, fs, tr	5 (9) ar, bu, fs, sd, tr	3 (10) fs, tr	1 (3) tr
Bolyeriidae	2 (2) fs, tr	0	0	0	0	2 (2) fs, tr	0	0
Tropidophiidae	2 (31) ar, sx, tr	0	2 (30) ar, sx, tr	2 (5) tr	0	0	0	0
Ungaliophiidae	2 (3) ar, fs	0	2 (3) ar, fs	1 (1) ar	0	0	0	0
Xenophidiidae	1 (2) sd, tr	0	0	0	0	0	1 (2) sd, tr	0
Caenophidia								
Acrochordidae	1 (3) aq, mr	0	0	0	0	0	1 (2) aq, mr	1 (2) aq, mr
Xenodermatidae	5 (16) sa, sd, tr	0	0	0	0	0	5 (16) sa, sd, tr	0
Pareatidae	3 (14) ar	0	0	0	0	0	3 (14) ar	0
Viperidae	44 (278) ar, fs, sa, tr	3 (19) fs, sa, tr	10 (71) ar, fs, tr	6 (45) ar, tr	3 (10) tr	10 (53) ar, fs, tr	24 (96) ar, fs, tr	1 (1) tr

(*continued*)

TABLE 4 (*continued*)

Taxon	*Diversity*	*North America*	*Middle America*	*South America*	*Europe*	*Africa*	*Asia*	*Australia*
Homalopsidae	11 (39) aq, fs, mr	0	0	0	0	0	9 (32) aq, fs, mr	7 (12) aq, fs, mr
Psammophiidae	7 (49) ar, tr	0	0	0	1 (1) tr	7 (44) ar, tr	2 (7) tr	0
Elapidae	69 (344) aq, ar, bu, fs, mr, sa, sd, sx, tr	3 (4) fs, mr, sd, tr	3 (28) fs, mr, sd, tr	2 (50) fs, mr, sa, sd, tr	0	10 (40) aq, ar, fs, mr, tr	22 (77) aq, ar, bu, fs, mr, sa, sd, sx, tr	48 (178) fs, mr, sd, tr
Atractaspidae	12 (71) fs, tr	0	0	0	0	12 (68) fs, tr	2 (3) fs	0
Lamprophiidae	33 (152) ar, fs, sa, sx, tr	0	0	0	0	33 (152) ar, fs, sa, sx, tr	0	0
Elapoidea *incertae sedis*	6 (22) fs, tr	0	0	0	0	4 (19) fs, tr	2 (3) fs, tr	1 (1) tr
Calamaridae	7 (82) sd, tr	0	0	0	0	0	6 (67) sd, tr	4 (16) sd, tr
Colubridae	114 (652) aq, ar, bu, fs, sd, sx, tr	24 (65) ar, fs, sd, tr	40 (172) ar, fs, sd, tr	18 (72) ar, fs, sd, tr	10 (18) ar, bu, sd, sx, tr	27 (116) aq, ar, fs, sd, tr	51 (295) ar, fs, sd, tr	7 (21) ar, tr
Pseudoxenodontidae	2 (10) tr	0	0	0	0	0	2 (10) tr	0
Natricidae	33 (205) aq, sa, tr	8 (37) aq, sa, tr	3 (32) sa	0	1 (4) sa, tr	10 (32) aq, sa, tr	15 (95) aq, sa, tr	4 (22) aq, sa, tr
Dipsadidae	93 (714) aq, ar, fs, sd, tr	7 (12) tr	51 (258) aq, ar, fs, sd, tr	62 (480) aq, ar, fs, sd, tr	0	0	0	0

a. Numbers of living genera and species (in parentheses) in each family, and total for the Serpentes.

b. Presence of species by continent: North America = United States and Canada; Middle America = Mexico, Central America, and the Caribbean; South America includes Trinidad; Europe = area west of 36° E (Arnold, 2002); Africa includes Madagascar and the Seychelles; Asia = area east of 36° E and west of Wallace's Line; Australia = area east of Wallace's Line, including New Guinea, New Zealand, and some Pacific Island groups.

c. Characterization of snake species in each family by primary habitats used: aquatic (aq) = in freshwater rivers, streams, lakes, ponds, marshes, and swamps; semiaquatic (sa) = at interface between aquatic and terrestrial habitats; terrestrial (tr) = on ground in forests, savannas, deserts, and other terrestrial habitats; surface debris dweller (sd) = in forest leaf litter, or beneath logs, rocks, and other surface debris; burrow dweller (bu) = occupy burrows they excavate or those excavated by other organisms; fossorial (fs) = burrowed beneath the ground surface in sand and loose soil; arboreal (ar) = above ground on bushes, tree trunks, or in the tree canopy; saxicolous (sx) = found on or among rocks; marine (mr) = in warmer waters of the world's oceans.

(e.g., kidneys, gonads), and others are reduced in size or lost (e.g., left lung, bladder). All of these traits reflect the trend toward a tubular-shaped body as compared to most lizards.

A change in the nature of body scales, which are usually arranged in oblique rows, has accompanied the shift toward elongation. The number of rows ranges from 10 to more than 90. Elongate and burrowing species tend to have fewer rows than do stout terrestrial or aquatic species (especially at midbody), as well as enlarged transverse ventral scales (except in scolecophidians, most alethinophdians, and some sea snakes whose body scales are of a uniform size).

Another trait that distinguishes most snakes from lizards is the use of olfaction as the primary sensory modality for locating mates and prey. Presumably, chemoreception was also important in the life histories of their fossorial ancestors. Morphological adaptations for olfaction in snakes include a deeply forked tongue used to "taste" the environment, a well-developed *Jacobson's organ* (a sensory structure used to interpret the sensory cues picked up by tongue flicking), and paired scent glands at the base of the tail. These glands produce a very bad-smelling secretion that may repel certain snake predators. The secretion also may be deposited as the snake moves through the environment, leaving behind a detectable chemical trail. The ability of male snakes to track females for considerable distances and through complex environments is well documented in the literature. The auditory capability of snakes, in contrast, is greatly limited. They have no external ear, and the middle ear is reduced. They hear by transmitting low-frequency vibrations from the ground through the lower jaw to the quadrate and columella and from there to the well-developed inner ear for interpretation. Vision also is important in snakes and is generally geared to detection of movement. Morphologically, the snake eye is unique among reptiles and seems to have evolved for nocturnal vision in a fossorial environment.

Snakes also have lower body temperatures than most lizards, and their tubular body shapes are thermally more efficient. As a result, snakes are able to reach optimal core body temperatures more quickly than lizards and usually without total body exposure to the sun. Except at cool northern or southern latitudes, diurnal snakes typically do not bask as do most diurnal lizards at the same sites. In arid habitats, where day time temperatures are often extreme, snakes are active in the cooler hours, and in some desert environments many are active only at night. Because the thermal exposure that snakes require to reach their preferred temperatures is reduced, their exposure to diurnal, sight-hunting predators (e.g., birds) is also reduced. On the other hand, less exposure also diminishes their opportunities to use visual displays in their daily activities. Given that most snakes also lack color vision, it is not surprising that they do not engage in the intricate, complex displays that characterize the social activities of many diurnal lizards. Male snakes typically do not operate in a social hierarchy, and neither brightly colored alpha males (e.g., as in the diurnal gekkos, *Gonatodes* spp.) nor vividly colored gravid females (e.g., some crotaphytine iguanids) are found among snakes. Likewise, the highly visible, stereotypic behavioral displays of many territorial lizards (e.g., head bobs, pushups, crest elevations, dewlap flashes) are not part of the behavioral repertoire of snakes. Sexual selection, which is such an important driving force in the life history of many lizards, is relatively inconsequential in snakes.

In contrast to lizards, all snakes are carnivores and eat a variety of invertebrate and vertebrate prey. Many snakes are generalists in their feeding preferences; others specialize on a specific type of prey. For example, there are snake species that feed primarily on each of the following groups: slugs and snails; earthworms; centipedes, scorpions and spiders; ants and termites; crustaceans; fish; frogs; lizards and other snakes; bird eggs; or mammals. These specialists often have morphological, ecological, and behavioral traits that reflect their diets. Ontogenetic shifts in diet occur in many species of snakes: the young eat invertebrates, and adults feed on larger prey, including vertebrates. However, the young of other snakes respond to prey size rather than type and simply eat smaller individuals of the same general group as do adults. Some species are able to kill and eat very large prey that sometimes exceeds their body size by 150 percent; they exhibit various morphological and behavioral traits associated with this preference (e.g., high number of body scale rows in larger constrictors, which increases the ability of the body to expand, and "sit and wait" foraging behavior accompanied by an effective venom delivery system in many vipers). Many morphological modifications promote safe and efficient prey capture and manipulation in the absence of limbs. For example, various components of the skull have been restructured to facilitate swallowing of large prey. Parts of the skull are loosely connected to the fairly solid braincase by ligaments and flexible joints that permit considerable distortion of the head. In addition, and unlike lizards, each half of the lower jaw of a snake can move independently, each mandible is jointed midway along its length, the teeth are recurved, the skin in the neck region is distensible, and neither a pectoral girdle nor a sternum is present. These modifications allow for an increased gape and facilitate the movement of large prey items into the gut with relative ease.

Some types of snakes simply seize and swallow a prey item. This is especially true of primitive scolecophidians, which are usually small, have a relatively rigid skull, and exhibit various tooth orientations and arrangements. They apparently feed exclusively on ants and termites (all stages, eggs to adults), which are clumped and abundant. Many species of scolecophidians live in termite mounds, whereas others forage widely and follow pheromone trails of ants.

In a second feeding mode, the snake grabs a prey item, encircles it with body coils until the prey is immobilized or dead, and then releases and eats it, often in a stereotypical fashion (e.g., always head first). These constricting snakes eat most of their prey with alternating movements of each side of the skull and jaws. The snake engages the prey with recurved teeth on one side and pulls the item posteriorly while releasing it on the other side, and then repeats this action from the opposite side, essentially walking its head around and over the prey. The anterior location of the tracheal opening allows the snake to breath with a mouth full of food, and the elastic nature of the skin and a high number of dorsal scale rows permit large items to be moved posteriorly into the stomach. Some constrictors (e.g., tree boas) ambush (sit and wait) their prey; these species often rely on visual (prey movement, mammals) and thermal (differential between prey and background temperatures, birds and mammals) cues to detect and capture suitable-size food. Other constrictors are active foragers and constantly search for suitable prey. The diurnal species often have large eyes and make use of both visual (i.e., movements of lizards, snakes, and birds) and olfactory (i.e., follow scent trails of mammals) cues. In contrast, active foragers that are nocturnal often have small eyes and seem to rely more on chemosensory cues to detect prey or the areas where they are active (e.g., runways, burrows, nests). Olfactory recognition and trailing often lead to prey that, depending on their size, the snake grabs and eats (e.g., nestling birds or baby mice) or grabs, constricts, and then eats (small mammals).

In a third and perhaps the most interesting mode of prey capture, the snake uses venom to immobilize its prey. Venom delivery systems are quite variable. In some species, modified salivary glands produce toxic secretions that pass into the mouth through a duct opening near the posterior maxillary teeth. In rear fanged snakes, venom flows into a wound through one to a few enlarged grooved posterior teeth. These venom delivery systems are relatively inefficient compared to that of the front-fanged snakes such as elapids and their relatives. In these forms, a hollow, immobile fang at the front of each maxilla is connected to a venom gland that uses special muscles to force venom through the hollow teeth into the prey. Snakes that fit into this third mode are mostly active foragers, and many feed on elongate prey, such as other snakes. The fourth type of venom delivery system is the most effective and is characteristic of vipers. In these snakes the maxilla is short, can rotate, and carries a single, long, curved fang that is folded against the roof of the mouth. During a strike, the maxilla and fang are rotated to a slightly forward position and thrust into the prey.

Snake venoms and their delivery systems evolved primarily to facilitate the capture and eating of specific kinds of prey. They immobilize and kill prey efficiently, with minimal ancillary damage to the predator. Snake venoms are either neurotoxic or haemotoxic, the former generally associated with elapids and the latter with vipers. Neurotoxic venoms have low molecular weights and spread rapidly in the blood stream; they generally lead to respiratory failure, and they work well on other snakes and lizards. Haemotoxic venoms are more complex and contain several digestive enzymes; because of their high molecular weights, they spread more slowly through the prey. These venom traits are especially useful to snakes feeding on large, bulky prey because the venom initiates digestion from the inside of the prey. At the same time, digestive enzymes in the snake's stomach initiate digestion from the outside. The dual action decreases the duration of the digestive period. Snakes can also use venom to defend themselves against potential predators, which often leads to conflicts between snakes and humans.

Obvious correlates of an effective venom delivery system are the various displays used by venomous snakes to warn predators of a potential defensive attack. Many of these behaviors are visual (e.g., the characteristic pattern on the hood of a cobra, the S-shaped threat display of a rattlesnake about to strike, and gape displays showing fangs of rear-fanged snakes), whereas others are auditory (e.g., the characteristic rattle of a rattlesnake). Perhaps the most obvious warning signals are the bright color patterns of the venomous species of New World coral snakes. The relationships between these *aposomatic patterns* and the presence of venom are well documented. Less venomous or nonvenomous species imitate, or mimic, the bright red, yellow, and black patterns of the coral snake models, thereby deceiving potential predators into thinking that they too are venomous and best avoided. The aposematic coloration of coral snakes, which has been mimicked by many other terrestrial, leaf-litter dwelling organisms (even including some invertebrates), has contributed impressively to the evolution of more species of Neotropical snakes. By virtue of the "protection" acquired from their venemous models, harmless mimetic snakes are able to enter adaptive zones and enjoy lifestyles that in the absence of protection from predators they might be too vulnerable to exploit. Aposematic coloration that announces the potential of a venomous encounter has been "adopted" in many mimetic lineages, increasing overall species richness and within-clade diversity.

Another obvious difference between snakes and lizards is the general absence of sexual dichromatism and body ornamentation in snakes. While striking patterns of dorsal coloration characterize many lizards and snakes, and geographical variation and pattern and color polymorphisms are well documented in species of both groups, fewer than 10 species of snakes have marked sex-related color dimorphism (e.g., the dipsadid *Manolepis putnami* is the only New World species), and only a few more have ornamental structures. For example, the dorsal crests, fins, dewlaps, and other structures that commonly adorn individuals, especially males, of many lizard species in several of the large groups of iguanian lizards (e.g., agamids, chamaeleonids, corytophanines) are rare in snakes. The nonvenomous arboreal Malagasy species of *Langaha* (see Fig. 48, in Chapter 10) and the aquatic Asiatic species of *Herpeton* are exceptions, having striking nasal appendages. Ocular spines are known in a few African, Asian, and North American sand-dwelling vipers (e.g., *Bitis, Cerastes, Crotalus*); they also characterize a few African arboreal (*Atheris*), Asian terrestrial (*Protobothrops*), and Neotropical arboreal (*Bothriechis, Ophryacus*) vipers. Prominent nasal spines are visible in the Rhinoceros Vipers (*Bitis nasicornis*) of southern Africa.

While there is little sexual dimorphism in snake coloration and pattern, most snakes are sexually dimorphic in size. Females often are larger than males, but many exceptions exist, especially among species in which males engage in ritualized combat and fight for mates. Females have longer bodies and shorter tails, presumably to maximize body space for egg storage and embryo development. Likewise, males often have longer tails to accommodate their hemipenes. In most species that have been examined, the numbers of *vertebral units* (i.e., total count of ventral and subcaudal scales with the anal plate as the landmark) in males and females are comparable within a population. This indicates that segmental dimorphism in most snakes reflects a shift in the position of the anal scale, rather than an actual difference in segmental counts.

All male snakes have paired hemipenes, and fertilization is internal. The hemipenes extend as sac-like structures posteriorly from the cloaca into the base of the tail in males. During courtship, the retractor muscle at their tip is relaxed, and the hemipenis fills with blood and lymph and everts out through the cloaca; the eversion is accompanied by contraction of the propulsor muscle. Following insemination, the propulsor muscle relaxes and fluid pressure is reduced; the retractor muscle contracts, and the organ is pulled back into the tail. The everted organ has a *sulcus spermaticus* (groove) that may be single or bifurcate and along which sperm travel during insemination. Hemipenes vary in size, shape (single, bilobed, divided), and ornamentation (spines, flounces, papillae and calyces); this morphological diversity is species specific and useful in classifications and for showing relationships (see Figs. 45 and 47 in Chapter 10).

Mating is usually preceded by a complex courtship that consists of a chase, stimulation, alignment, and intromission. Once the sex of the individual is determined, tactile rather than visual stimulation occurs; it includes intertwining, head rubbing, and sometimes biting of the female's neck. During the alignment phase, the male brings his tail into contact with the female's tail, initiates a series of caudal muscular contractions, and everts a hemipenis. In the final phase the female opens her cloaca, the male inserts a single hemipenis, and insemination occurs. Fertilization occurs subsequently in the oviduct, before the eggs acquire their shells.

Like lizards, most snakes are polygynous. Reproductive activity for temperate species that hibernate is often correlated with times of emergence from dens. In tropical regions, reproduction may occur at any time but is often synchronized with the rainy season. For some species this may be the time when environmental conditions for egg or embryo development are best; for others it may be a time of high prey densities and better foraging for the females or the young. While courtship and copulation may be tractable, fertilization often is not. Females of many species are able to store sperm in their oviducts for relatively long periods, and fertilization of the eggs may occur weeks, months, or even years after copulation. Also, in some tropical species mating occurs shortly before ovulation; in colder climates or at higher elevations, the period between these events may be longer. Finally, the reproductive cycles of males of some species may be tightly synchronized with those of females at a site, but males of other species at the same site may be reproductively active all year. Clearly much remains to be learned about the reproductive cycles of most tropical species.

Most snakes are oviparous (lay eggs), but some are viviparous and give birth to live young. Snake eggs are usually broadly oval, but some are narrow and more elongate. All are covered by a smooth or granular shell that is permeable to gases and water. Some eggs are covered by an oviductal fluid and stick together when laid. All eggs take up water from their surroundings and swell, and moisture content appears to be a critical component of a suitable incubation site. Deposition sites are characterized by warm temperatures and moderate humidity, with little fluctuation, but they vary among species. Snake eggs are frequently found in piles of decaying leaf litter, rotting hollow logs, insect and other burrows, sand or friable soil, and termite nests. A few species build nests of decaying vegetation or make nests in the ground, and some species guard them. Some pythons coil around their eggs to protect them or maintain a relatively constant incubation temperature. Clutch size generally varies by species and by female age, size, and condition, with smaller species having small clutches (2 eggs) and large species having much larger ones. Most clutches number fewer than 50 eggs, and numbers between 7 and 15 are common. Some scolecophidians lay as few as 2 eggs, and large clutches (>150) have been reported for an African viper. Temperate species tend to have larger clutches than tropical forms, and more offspring at more northern latitudes and at higher elevations. Most species reproduce once a year, but a few tropical species have been reported to breed twice a year, and a few others (*Acrochordus* spp.) as infrequently as once a decade.

Snake activity and movements are generally associated with feeding and predator avoidance, although some other activities (e.g., thermoregulatory behavior, searches for protected sites for egg laying or skin shedding) are also important. In temperate regions, snake activity may be temperature-dependent and bounded by movements to and from winter *hibernacula.* At certain times of the year, snakes may be extremely abundant (e.g., as they emerge from hibernacula, beneath cover objects in open habitats in the spring, in certain meadows) in some temperate areas. In tropical regions, snakes may be active throughout the year, but the activity patterns of some species are correlated with seasonal changes in rainfall or the seasonal availability of prey (e.g., frogs). As a result, activity patterns of wide-ranging species of tropical snakes may vary geographically. It is also possible that the wet and dry seasons of many tropical forests temporally reduce the availability of suitable egg-laying sites in tropical soils or decaying litter, restricting breeding cycles accordingly. Even though the species diversity of snakes at tropical forest sites may be three to four times greater than those at comparable temperate sites, snakes in tropical forests are relative scarce. Finding more than three snakes during 6 hours of nighttime searching along forest trails in Neotropical lowland forest is unusual. It may take four times the number of sampling days to complete an inventory of the snake fauna at a site than it does to determine lizard or frog diversity at the same site. Why are snakes so difficult to find, and why are they inactive so much of the time? Much remains to be learned.

Snakes face many of the same threats from human population growth as do other reptiles, but generally hunting pressures to supply skins for the commercial trade have been less intense. Habitat modification and loss, together with the impact of introduced predators and populations of feral pets, remain the primary problems for snake populations, especially on small tropical islands and in urban regions. However, because some species of snakes produce venom that can be fatal to humans and are able to deliver it efficiently, humans tend to fear snakes and kill them whenever they are encountered, even when they are nonvenomous. Den sites are especially vulnerable to programs of extermination (see Chapter 16, “Monitoring Exploited Species”). The impact of such programs in the United States, especially in the northeast and in Texas, has been substantial. Unfortunately, fear of snakes continues to plague the human psyche.

General references that are especially informative include Greene (1997), Shine (1991a), and Vitt and Caldwell (2009). Many regional treatments also provide useful information on snakes: for North America, see Conant and Collins (1998), Gibbons and Dorcas (2005), Stebbins (2003); for Middle America, see Campbell (1998), Campbell and Lamar (2004), Crother (1999), Henderson and Powell (2009), Köhler (2003), Köhler et al. (2006), Lee (1996), Liner and Casas-Andreu (2008), Powell and Henderson (1996), Roze (1996), Savage (2002), Schwartz and Henderson (1991), Solórzano (2004), Stafford and Meyer (2000), Wilson et al. (2010); for South America, see Amaral (1977), Campbell and Lamar (2004), Carreira et al. (2005), Cei (1993), Duellman (2005), Giraudo (2001), Murphy (1997), Pérez-Santos and Moreno (1988, 1991), Roze (1996), Starace (1998); for Europe, see Arnold (2002), David and Vogel (2010), Sindaco and Jeremčenko (2008); for Africa, see Bons and Geniez (1996), Branch (1998), Chippaux (1999), Dobiey and Vogel (2007), Glaw and Vences (2007), Goodman and Benstead (2003), Le Berre (1989), Pauwels and Vande weghe (2008), Spawls et al. (2002); for Asia, see Alcala (1986), Anderson (1999), Chan-ard et al. (1999), Cox et al. (2010), Das (2004, 2010), David and Vogel (1996, 2010), de Lang and Vogel (2005), Disi et al. (2001), Khan (2002), Murphy (2007), Nguyen et al. (2009), Sindaco and Jeremčenko (2008), Somaweera and Somaweera (2009), Stuebing and Inger (1999), Vogel (2006), Whitaker and Captain (2004), Ziegler (2002); and for Australia, see Bauer and Sadlier (2000), O’Shea (1996), Wilson and Swan (2003).

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PART TWO

PLANNING A DIVERSITY STUDY

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CHAPTER THREE

Study Design and Sampling

ROBERT N. FISHER and MERCEDES S. FOSTER
Chapter Coordinators

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Overview

Robert N. Fisher and Milan Mitrovich

Our goal in this chapter is to help investigators lay the groundwork for design of their biodiversity studies. Study design follows no hard rules, and often even the best planned study has to be modified over the first few days of a project to accommodate unforeseen logistical complications. Although over-engineering a reptile study can delay initiation of the work and increase costs, under-planning often wastes valuable field time and results in unusable data. A carefully planned approach that is flexible in implementation and builds on others' successes is always the best approach.

Sometimes the most logical assumptions made in an office or laboratory turn out to be unrealistic in the field, and having "plan B" and even "C" ready to go will save time and money. Thinking things through beforehand and recognizing the uncertainty associated with conducting field investigations are vital for success. In this chapter we pose a variety of questions that one should ask during the design phase of a research project. These questions will not be the only ones that are important nor necessarily the right ones for a particular study; however, we hope that they will cause investigators to think about the questions most relevant to their own studies and to develop the most robust design possible in the face of biological, political, geological, and fiscal uncertainty.

Over the last decade the thought that has gone into designing inventory and monitoring projects has increased significantly, leading, for example, to the development of standardized protocols, records of metadata, use of more sophisticated statistical designs, and efforts to maximize signal in the resulting data. Consequently, the value of such studies for science and management should be more robust. Unfortunately, logic and common sense sometimes get lost, and investigators do not always employ the simplest study design and sampling strategy needed to answer the study question. Nevertheless, researchers should not assume that our suggestions for a study design are the only approaches to consider; rather, our goal is to help investigators to recognize and follow the logical steps needed to design their studies of the targeted taxa in their specific ecological settings. Many general books are available on study design (e.g., Sutherland 1996; Margoluis and Salafsky 1998; Elzinga et al. 2001; Morrison et al. 2008); therefore, we focus on the design of reptile studies.

The first step in any study is to articulate clear, obtainable objectives. The objectives and methods to be employed should be discussed with the land management agency, private individual, or other entity associated with the potential study site to ensure that they are consistent and/or compatible with the plans, mandates, or permit requirements of the area. Land management agencies include such entities as state or national park administrations, the forest service, village or tribal governing bodies, or a country. Each one could potentially restrict a study and should be dealt with upfront. Investigators should select study sites that are consistent with the research objectives. In the following sections, we discuss selection of approaches appropriate to the research. Before fixating on species, sites, or techniques, an investigator must know the true purpose of his or her study and identify and clearly define its objectives. The study objectives (and funding constraints) will dictate the study design, the data gathering techniques to be used, and the sampling regime.

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Scope of the Study

INVENTORY VS. MONITORING

Often, researchers develop monitoring plans without sufficient knowledge of habitat use by or spatial distributions of the populations they wish to target. Many times species lists for the target area are antiquated or have been developed from inadequate sources. In such cases, an inventory should be the first step of the study. An inventory allows the investigator to try novel techniques, assess unique habitats, and test assumptions based on previous lists or knowledge of the site. He or she can also use the inventory results to determine species richness by habitat type and study site. Once the baseline data are available, the objectives and targets of a monitoring plan can be formulated. For example, a planned monitoring program might include all species in the area, or it might be confined to unique species or rare habitats recorded during the inventory. In the latter instance, a suitable study design would call for techniques and sampling schedules appropriate for the targeted species or habitats and would include stratification and replication. If a study site is part of a series of management units (e.g., national parks or wildlife refuges), the investigator should assess the inventory and monitoring needs of many similar units to reduce redundancy in technique efficacy testing and to increase comparability of data across units.

QUALITATIVE VS. QUANTITATIVE SAMPLING

Depending on the objectives of a study, either a qualitative or quantitative approach may be valid. If the goal is to assess the biodiversity of a large tract of habitat rapidly and resources are limited, then a qualitative approach may be most appropriate. A team could build a species list for several sites without stratifying sampling across habitats or equalizing sampling efforts across sites or subsites and employ multiple sampling techniques without documenting effort per technique. The resulting species list would include from 20 to 100 percent of the possible taxa, but no assessment of the accuracy of the list would be possible. This result might meet the goal of the study, with the documented taxa now being part of the knowledge for the study site. Future work could be designed using the baseline data, but the lists themselves would not be statistically comparable across the sites surveyed.

Quantitative sampling usually requires a greater prestudy knowledge of the study site(s) and species to be targeted. The study objectives need to be clear so that appropriate sampling techniques and spatial and temporal scales of coverage can be identified before the study begins. This information needs to be recorded explicitly as metadata for the study so that future efforts and changes in protocol can be clearly tracked and tied to either the data analyses or replication of the study later on. Data collected in a quantitative fashion with documented protocols and metadata can be compared or combined across study sites and management units so that habitat use, activity patterns, phenology, population declines or increases, species' invasions, and/or a variety of other ecological factors can be analyzed in a statistically rigorous fashion. Furthermore, with appropriate documentation and prestudy data, others will be able to apply the study design and techniques to their target sites. Aggregation of the data for a regional analysis increases the value and power of the data from all the study sites.

Legacy data (i.e., datasets available from previous and likely unrelated field studies) from a site may allow for future quantitative comparisons if detailed descriptions of the techniques used to collect the initial data are available. If the techniques used are not fully explained and subsequent investigators must make assumptions about the integrity of the data, then they should use a conservative approach, limiting the amount of information they extract from the data. If this type of data is obtainable and approached in a conservative fashion, then very powerful statements about species' distribution and abundance changes over time can be made with statistical rigor (Shaffer et al. 1998).

TARGET SPECIES

Biodiversity studies can range from those with identified target species to those concerned with the entire pool of species known from a site. Sometimes a target site will lack any reptiles, but without a rigorous sampling regime, an investigator may not be able to detect this. Typically, studies target a suite of species, such as all lizards, aquatic turtles, or fossorial snakes. They also may target a single species, such as Gila Monster (*Heloderma suspectum*), or all species of amphibians and reptiles for a park, which could still be only a single species as is the case of Denali National Park in Alaska (*Lithobates sylvaticus*).

If habitat relationships among multiple species at a site are known but detection techniques vary across species, then an investigator can select a single species to serve as an *indicator* for a particular habitat and for the other known taxa that occur in that habitat but that the sampling technique used does not directly target. For example, investigators working in southern California found that the Striped Racer (*Coluber lateralis*) was always present in coastal sage scrub if the Orange-throated Whiptail (*Aspidoscelis hyperythra*) was present (Atkinson et al. 2003). *Coluber* numbers were always about one-tenth the values for *A. hyperythra* and often were insufficient for trend analysis. In contrast, *A. hyperythra* was easy to track, and large numbers of individuals could be detected rapidly in some instances (Atkinson et al. 2003). Consequently, this lizard species would seem to be an appropriate indicator for the snake. Apparently, if the snake occurs at a site, it can always be found if enough effort is expended. Using the lizard as a proxy for the snake is far easier (Atkinson et al. 2003).

Sentinel species signal the presence of certain, often negative, environmental conditions by reacting to them. They may die, get sick, leave the area, or experience a decrease in their reproductive success. An indicator species can also be used to assess the status of suites of species. An *umbrella species,* for example, is one that has an extensive home range or occupies a broad spectrum of habitats and that is known (or assumed) to have a relationship with a variety of other nontarget species at a site. The implication is that as the abundance of the umbrella species increases or decreases, the abundance of each nontarget species changes in the same fashion. Consequently, any action (e.g., management procedure) or event that affects the umbrella species is assumed to affect the larger pool of species as well. *Flagship species* are (generally) large, charismatic species, such as pandas, gorillas, and large cats (e.g., jaguars), with which members of the public tend to develop a special emotional bond; as a result of that bond, they support protection of the habitats required by the animal and in turn protect other, less attractive and noticeable species that occupy the same habitat. *Keystone species* are those that play a critical role in maintaining an ecosystem despite their low numbers and low total biomass in that ecosystem. Often, their wide-reaching effects are noted only after they disappear from a community that subsequently collapses. By monitoring keystone species, one can monitor the health and stability of an entire ecosystem.

Study Goals and Objectives

Before an investigator can define clear, obtainable research objectives, he or she must be able to articulate the desired goals of the research or the specific purpose of the study. For example, if a researcher undertakes a study to generate information upon which a management decision concerning a particular species, habitat, or ecosystem can be based, then he or she should be able to describe the specific management problem or question being addressed. By clearly defining the goals of the research early in the planning process, an investigator can formulate research questions or objectives that directly address those goals. In turn, the goals will influence the choice or development of specific methodologies to be employed and increase the likelihood that the goals will be met.

Although allowing the goals of a research project to determine the objectives and methodologies of the study appears obvious, inexperienced researchers rarely employ this approach. Instead, novice researchers often become fixated on specific research methodologies during the planning phase of a project and spend little time developing research objectives or exploring their relationships to an often poorly defined set of research goals. Without well-defined goals, researchers cannot critically assess the likelihood that their research will be informative (i.e., of value) in answering the identified applied and/or basic research questions of interest.

Researchers will be better prepared and more likely to develop cohesive research goals and objectives if they consult the primary literature and speak to local experts about the general ecology and natural history of the target reptiles prior to initiating a study. In that way they can acquire current knowledge specific to local populations of the target reptiles and be better prepared to identify knowledge gaps and target existing data needs more specifically. Researchers will also develop a greater awareness of the field and lab methodologies available to them when deciding how to meet their specific research objectives and goals. Researchers working closely with land managers, agency biologists, and conservation professionals early on in the development phase will strengthen the connections between their research and the needs of resource managers.

Developing an Effective Sampling Strategy

Research questions, developed in conjunction with the research goals, need to be translated into a specific action plan with a set of clearly obtainable objectives. During this process, research questions should be formed into *hypotheses,* or specific statements of the relationships between two or more measureable variables, that are testable by statistical and analytical means (Yoccoz et al. 2001). To facilitate testing, the expected relationship between the variables should be precise, limited, and stated in absolute, quantitative terms. Likewise, the replication of independent samples from the target population or populations is critical to being able to test the research hypotheses. Replication provides a basis for confidence in the estimates obtained and minimizes the noise of local effects. Reliable statistical inferences can be expected only when sample sizes are reasonably large and obtained randomly. An insufficient number of samples will lead to low confidence in the estimates obtained and provide little power to detect actual differences in the characteristics of sampled populations.

Once the hypotheses are defined and the geographic scale over which the results should apply is determined, the investigators select the techniques or methodologies they will use to collect the data. By using standardized techniques, the value of the research results is increased as studies using similar field protocols are often comparable across space or through time. Development of alternative sampling strategies will allow researchers to build maximum flexibility into their study design, so that their plans can be adapted to changing conditions and uncertainties encountered in the field.

Because of the need for replication and the likely reality of limited resources (e.g., time, money, personnel, equipment), researchers should collect their data during the period likely to provide the greatest return (i.e., samples or data points) per unit effort (e.g., trap-night, walking-survey). Studies must be scheduled when the focal species are most active, be it a time of year, time of day, or under specific weather conditions. Studies done at these times are most comparable among sites in addition to providing the greatest amount of data.

It is also critical for researchers to identify which of their specific research questions are most critical to the success of the overall project and to prioritize them before initiating the work. Researchers will then be able to make difficult decisions concerning which sampling strategy to adopt, especially if the investigator has underestimated the amount of fieldwork required to collect the necessary data. A nested set of research questions and/or plans provides a researcher with flexibility in the field, guarantees productivity, and ensures that a core set of questions will be answered, even if the work turns out to be more difficult than anticipated.

In the end, a researcher can be confident that he or she has designed a robust research project if all of the applicable questions listed in Box 1 can be answered successfully. The absence of answers for many of these questions likely indicates a deficiency in the study design and signals that additional planning is needed.

The Value of Long-Term Monitoring

J. Whitfield Gibbons

The contrasts between inventory and monitoring or between qualitative and quantitative sampling are greatest when short-term inventories (e.g., localized, single-season efforts) are compared with long-term monitoring studies that have been conducted over many years with a broad regional emphasis. Long-term monitoring can be particularly important in studies with reptiles because of the annual variability and unpredictability in apparent abundance of most species in response to seasonal weather conditions. Thus, a short-term inventory may not reveal the presence of a reptile species in any single year or even several, although the species is ultimately found to be locally abundant following long-term monitoring efforts. Long-term monitoring may successfully reveal that a species is present because of timing (monitoring eventually was conducted during a year when the species was apparent), spatial extension of sampling efforts (because of continued monitoring, new habitats were explored), or because of the use of different trapping methods that had not been used earlier. For truly rare or highly secretive species for which observations are usually serendipitous, long-term monitoring will

BOX 1 QUESTIONS IMPORTANT TO CONSIDER WHEN PLANNING A BIODIVERSITY STUDY

- What is the purpose of the study; what are its goals?
  - To inform management actions?
  - To support conservation efforts through systematic monitoring or a one-time biological assessment?
  - To inform resource agencies concerning the conservation status of a single species or community?
  - To assess the distributional limits of a particular species or community?
  - To improve understanding of wildlife–habitat relationships?
  - To further advance basic knowledge concerning the ecology of a particular a species or community?
- What are the objectives of the study?
  - To determine what species are present (i.e., occurrence)?
  - To determine where each species is found within the study area (i.e., distribution)?
  - To determine which habitat each species occupies (i.e., habitat–species relationships)?
  - To compare community structure between areas (i.e., composition)?
  - To measure the population size (relative or absolute) or proportion of area occupied of each species within the study area?
  - To compare the relative abundance and/or population structure of each species between areas?
  - To measure reproductive success, survivorship, dispersal, or apparent recruitment of each species?
- What is the nature of the study?
  - A one-time or initial baseline survey (i.e., an inventory) that examines the status of a species or community (a snapshot in time)?
  - An ongoing monitoring program to determine population trends? To measure how conditions (e.g., species richness, species distributions, abundances, recruitment, reproductive successes, population structures, habitat–species relationships) of a population/community have changed through time?
  - A series of surveys that examine the causes of observed changes: species-stressor relationships, species–habitat relationships, species-management action relationships?
- What resources are available for the study?
  - What are the financial constraints/opportunities associated with the project?
  - What are the logistical constraints/opportunities?
  - How have time and personnel needs been addressed?
  - Background information about the organism(s) of concern?
    - What species are potentially present?
    - What type of information is available from background literature, museum searches, and local experts?
- What are the bounds of the study?
  - What is the geographic scope?
  - What is the temporal focus?
  - What are the target species or populations?
  - At what level should the inferences apply?
- What are the sampling considerations?
  - What are the assumptions of the sampling scheme?
  - What protocols will be used?
  - How will species absence be determined?
  - Where and when will sampling occur? How will the influences of season, time of day, weather, habitat, and so forth, be incorporated into the study design?
  - How will quality assurance be maintained?
  - How will the influences of body size, age, gender, and life stage be incorporated into the study design?
- How will the data be managed?
  - What are the strategies for long- and short-term storage?
  - What are the plans for the analysis of data?
  - How have decisions concerning data analysis influenced the study design?
  - How will the results be presented?
  - What are the expected products of the work?

be more likely to reveal a species' presence in an area simply because of the increased opportunities for encounter.

Short-term, low-intensity monitoring also warrants comparison with long-term monitoring. Although short-term monitoring of population features, such as size and age structure, may reveal important information about the current demographic status of one or more species, a limited temporal effort cannot verify the long-term trends in population size or health that are necessary for prudent management or conservation plans. Long-term monitoring may be the only accurate way to assess certain population traits of target species in a region. Thus, population declines of some reptile species may be difficult to detect and document without long-term monitoring of natural populations and communities, a study approach that has been considered crucial for understanding population status and trends of a variety of animal species, including reptiles (Tinkle 1979; Cody 1996; Gibbons et al. 2000). In fact, because most field studies of reptiles have not been of the depth, intensity, and consistency characteristic of long-term monitoring, few credible determinations have been made of the population status and fluctuations over years for one or more reptile species (Pechmann and Wilbur 1994; Gibbons et al. 2000). Resolving whether observed population trends represent fluctuations that are normal for the species or whether they are reflective of unnatural declines in response to anthropogenic activities may be difficult or impossible without effective long-term monitoring. Distinguishing between natural and human-caused declines (or increases) in wildlife populations is important from management and conservation perspectives, as long-term studies have documented that some reptile populations can vary greatly across years due to natural causes unrelated to human activities (Congdon and Gibbons 1996; Vitt et al. 2003).

How Long Should Long-Term Monitoring Continue?

One of the problems with any type of monitoring program is that no matter how long a study has been conducted, continued monitoring will probably reveal patterns not previously observed. Those new patterns often lead to different interpretations of the environmental system or the role of the target organisms in it. Ash (1997), for example, reached a conclusion about the effects of clearcutting on terrestrial salamanders that differed from the conclusion of Petranka et al. (1993), whose study was shorter. Although the length of the monitoring program was not the only difference between the two studies, the strength of long-term monitoring adds to the credibility of some findings. Long-term approaches can be especially valuable for reptile studies in which the animals themselves are likely to live for a long time, so that the same individual animals experience different environmental conditions over time. Setting an exact period that is appropriate for monitoring a particular species, species grouping, or habitat cannot be done effectively. The unpredictability of inconsistencies in annual weather affects both the apparent and actual abundance of many species of reptile; hence, the absolute length of a monitoring program in one set of years may differ dramatically from another in which prolonged drought or flooding prevails. Long-term monitoring efforts can be quantified during the course of a study or at the end, but projecting the number of years of monitoring that will be necessary to achieve certain goals is for the most part guesswork. Thus, 5 years may serve as a standard in some situations, even though 3 years could be sufficient for reaching some goals and 7 years might be too short a time for reaching other goals.

Short-term studies of reptiles can provide important and useful information about species, especially when monitoring is conducted in a diversity of habitats and in different geographic regions so that comparisons can be made. Nonetheless, certain insights about reptile populations are unavailable with short-term monitoring and can only be obtained from long-term monitoring. Thus, the strongest support for certain conclusions in some situations, such as whether a population decline has occurred in a species in a particular region, is best acquired by means of long-term data sets that have registered population levels for target species of reptiles in restricted habitats or specific locations.

Examples of Long-Term Monitoring Studies

Several examples exist of long-term reptile studies that have provided information about particular species that would not have been obtained even with multiple short-term studies conducted in different study areas. Numerous long-term research studies have been conducted with turtles (e.g., Iverson 1991; Frazer et al. 1993; Congdon and Gibbons 1996). Below, I provide examples of studies in which information on declines of particular species was obtained through basic monitoring of population numbers over many years.

Garber and Burger (1995) concluded that a New Jersey population of Wood Turtle (*Glyptemys insculpta*) had in essence been eliminated because of public-access issues that included capture of individual turtles as pets, road mortality, mortality from dogs accompanying visitors, and the attraction of wild predators to food disposal bins. The documentation of the steady decline in the turtle population was possible because of a 20-year study.

Another long-term monitoring study of a turtle population was conducted on Capers Island, South Carolina, a barrier island where individual adult Pond Slider Turtles (*Trachemys scripta*) were censused on a near-annual basis from 1978 to 1990 (Gibbons et al. 1979). Juvenile recruitment did not occur, because of documented nest predation on land and presumed predation by alligators in the water. However, although most adult turtles were too large to be eaten by the majority of alligators, demographic analyses revealed a slow but steady decline in the turtle population due to loss of the large adults from unknown causes. Confirming that a natural decline was occurring in the isolated turtle population would not have been possible without long-term monitoring.

Annual monitoring of the numbers of Diamond-backed Terrapin (*Malaclemys terrapin*) in a series of tidal creeks along the Kiawah River in South Carolina revealed that population numbers had declined steadily from the onset of the study in 1984 until 2004 (Gibbons et al. 2001; Tucker, Gibbons, and Greene 2001; Dorcas et al. 2007). It also linked the decrease in terrapin numbers to the increase in recreational crab trapping (Hoyle and Gibbons 2000).

That the numbers of sea turtles have declined in most parts of the world has been demonstrated convincingly for several species because of the long-term monitoring efforts on nesting beaches at numerous locations. Based on 16 years of data from nesting females, Spotila et al. (1996) estimated that the worldwide population of the Leatherback Sea Turtle (*Dermochelys coriacea*) in 1980 numbered more than 100,000 individuals, compared to fewer than 35,000 in 1995. Hildebrand (1982), after monitoring nesting females from 1947 to 1975, concluded that the numbers of Kemp's Ridley Sea Turtle (*Lepidochelys kempii*) at the species' largest nesting site in the Gulf of Mexico had decreased from more than 40,000 individuals to fewer than 1,000.

The disposition of regional faunas or faunal communities can also benefit from long-term monitoring. Surveys from 1978 to 1998 in the Snake River Birds of Prey Area reported by John Cossel, Jr. and Charles R. Peterson (Gibbons et al. 2000) revealed that reptile species numbers at two dozen sample sites had decreased by an average of 5 percent. Such subtle declines typically cannot be detected or confirmed without long-term monitoring efforts and surveys that provide population estimates over many years.

Many of the examples given above provided evidence necessary to suggest the causes of the observed declines. Without long-term monitoring efforts, declines may go unnoticed, resulting in the conservation dilemma that "once an unmonitored species or population is recognized as being unexpectedly depauperate or absent, the cause of decline may be unknown and unknowable" (Gibbons et al. 2000).

Conclusions

Gibbons et al. (2000, p. 662) summed up the importance of long-term monitoring as follows:

> [L]ong-term monitoring of reptile populations is essential and must be aided by the establishment of standard methods and techniques. It is equally important that the academic community, land managers, and conservation organizations recognize that rigorous field programs focusing on the distribution, abundance, status, and trends of populations and species are critical and worthwhile. Herpetofaunal inventories should become a standard part of environmental assessment programs, and the publication of field survey efforts that document potential or suspected declines should be encouraged. When long-term and widespread monitoring becomes the norm, declines are likely to become less equivocal (in terms of protracted declines versus natural fluctuations) and the causes less mysterious.

Ultimately, that will give managers and conservationists a greater likelihood of reversing the declines.

The fossorial habit and secretive nature of many long-lived species of reptiles, the relatively large home range sizes of some species, and the often low population densities limit the ability of short-term monitoring programs to document population trends, which may vary over periods of years. The first step in applying effective conservation measures to address declining reptile populations is to identify the problems. In many if not most cases, long-term monitoring programs will be necessary to do so.

Acknowledgments

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Know Your Organisms

Joseph C. Mitchell

Identification and Natural History

Proper identification of organisms is an absolute requirement for a successful and, indeed, useful inventory or monitoring project. In fact, Sutherland (1996) included "not knowing your species" as one of the 20 most common censusing sins. Reptiles are notorious for exhibiting ontogenetic, sexual, and geographic variation in phenotypic characteristics. Different species are active at different times of the day and in different seasons. Juveniles may occupy different habitats from adults, and habitat use may shift with season. The abundance of many animals, especially snakes, is easily underestimated because they are so secretive. Unless the natural history of the target organism is considered when developing a study design, even the best techniques and statistical protocols can produce completely erroneous results.

Inexperienced people, including herpetologists working in new areas, often misidentify specimens because of phenotypic variation within reptile species. Variation within single populations can also be problematic with some species. A single population of the Neotropical Eyelash Viper *Bothriechis schlegelii,* for example, can exhibit several distinct color phases within the same population (Campbell and Lamar 2004). Many snakes and some lizards and turtles exhibit ontogenetic variation. Eastern Ratsnakes (*Pantherophis alleghaniensis*) and North American Racers (*Coluber constrictor*) in eastern North America are gray with dark blotches as hatchlings, but they are completely black as adults (Fig. 2). Juvenile Great Plains Skinks (*Plestiodon obsoletus*) are completely black, but adults are brown with dark-tipped dorsal scales suggestive of stripes. Sexual dimorphism in color and pattern occurs in several species of lizards (Fig. 3), especially during the breeding season when hormone levels influence coloration (e.g., Leopard Lizards, *Gambelia*; Lesser Earless Lizards, *Holbrookia*; Toothy Skinks, *Plestiodon*; Spiny Lizards, *Sceloporus*). Geographic variation in color and pattern is also common in many species of reptiles. Eastern Ratsnakes in southeastern North America, for example, are yellow with dark stripes, whereas those in the northeast are completely black. Striped Mud Turtles (*Kinosternon baurii*) at the northern end of their range do not have stripes. Researchers and their assistants should be familiar with all forms of variation exhibited by the reptiles in their study areas.

All field personnel must be able to identify all of the reptile species expected for the study area correctly. They can do several things to increase their knowledge of local reptiles, which can significantly increase the quality of the project. The important first step is to consult field guides and primary literature to develop a list of species likely to occur in the geographic area and habitats to be inventoried or monitored, and then to study the phenotypic and ontogenetic variations of each one. If necessary, investigators can prepare identification keys or personal field guides. Learning the basic taxonomy of the species groups, as well as which ones have named subspecies and where the nearest zone(s) of intergradation is located, is helpful, as is knowing whether species that closely resemble each other but are recognized as separate species are present. All field personnel should receive training to ensure the quality of the information contained in their notes.

Knowledge of the natural history of the target species should be incorporated into the original study plan. Such information facilitates decisions about the habitats to be studied and the optimal timing of sampling both seasonally and during 24-h cycles. Knowing the movement patterns and behavior of the target species and the techniques best suited to that behavior and to the combination of day, season, and habitat will increase the encounter rate with the focal species. Knowing which species will have to be captured to ensure proper identification is also important because it will allow the investigator to select the least disruptive capture technique. Accurate answers to

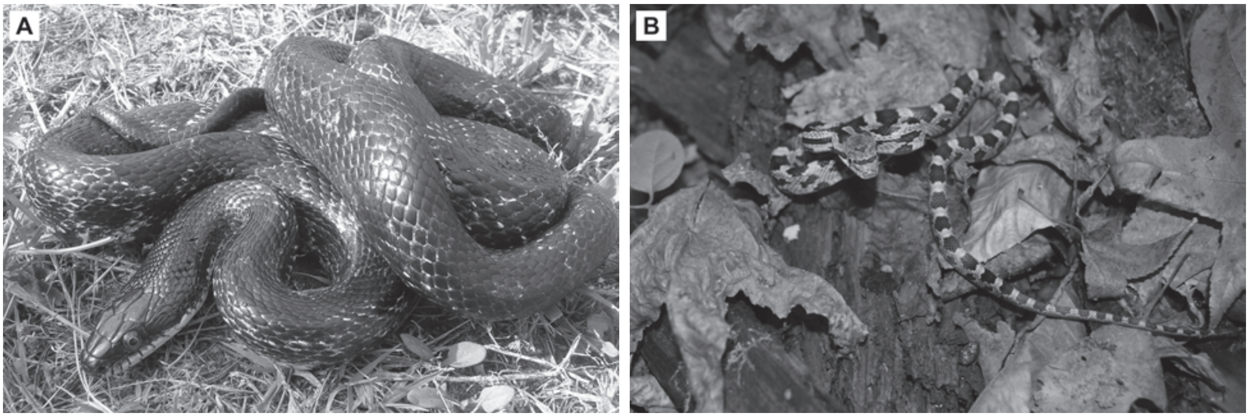


FIGURE 2 Ontogenetic variation in Eastern Ratsnakes (*Pantherophis alleghaniensis*). (A) Adults in the northern portion of the range are almost completely melanistic. (B) Hatchlings and 1-to-2-year-old juveniles have a blotched pattern on a gray background that fades with age. (Photos by J. C. Mitchell.)

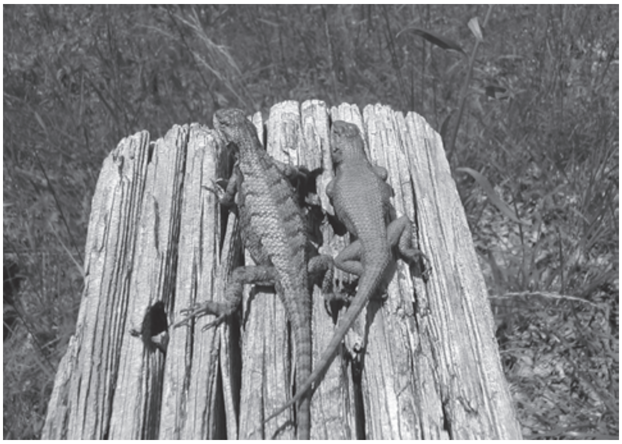


FIGURE 3 Sexual dimorphism in Eastern Fence Lizards (*Sceloporus undulatus*). The adult male (right) has blue patches on the throat and venter and lacks a dorsal pattern. The adult female (left) possesses prominent dorsal crossbars, lacks blue pigment, and is usually larger than the male. (Photo by J. C. Mitchell.)

questions about a species' behavior and phenotypic variation are possible only when one understands the basic natural history of the species to be monitored. Researchers can waste enormous amounts of effort and funds if they use inappropriate field techniques and sample at the wrong times and/or in the wrong places. Although an increasing number of useful books on reptiles are available for some regions, such as Australia, Europe, North America, and parts of South America, Africa, and Asia, other regions lack such summaries. How does one obtain critical information on identification and natural history of the species in a study area if such resources are not readily available? Under such circumstances, investigators should consider conducting an initial inventory of reptiles in the area before beginning the monitoring phase of the study. Fieldworkers can record activity times, habitat use, and behavior of different species and obtain voucher specimens for museum collections. Once a synoptic collection has been accumulated and studied, then the researcher can prepare checklists, keys, and a reference collection for use by field personnel. These resources will help ensure the accuracy of species identification in the field.

Training Observers

Researchers experienced with the species or species groups under study should have few problems with the many aspects of identification. However, in numerous situations a project manager or researcher must use students, volunteers, or other persons to execute the project. These people come with varying levels of experience and knowledge, from none to a good understanding of the local species and their variations. Training of students, volunteers, employees, and subcontractors is an essential part of a successful project.

An important responsibility of a project manager or primary researcher is to ensure that the field personnel work together as a team, using their talents to achieve common goals in an efficient manner. This is not always easy, because it involves training people with varying levels of experience, keeping them motivated, and getting them to work together. Experience and knowledge can be enhanced in numerous ways that involve training in some fashion. Fellers and Freel (1995) provided a useful overview of basic qualifications for field biologists, training, project supervision, and quality assurance. They also included an outline for a training session and criteria for evaluating field personnel. Although they focused on amphibian monitoring, the information they presented is easily adapted to projects that focus on reptiles. Project heads can use several tangible methods to bring all field personnel up to a common level of knowledge. They can provide field guides, other written materials, and specimens to aid the learning process; require employees to study them and carry them in the field for reference; and devise tests to determine how well personnel know the species they will likely encounter. Depending on the number of individuals involved, the project head may want to offer a "mini training course" that addresses the specific skills required to meet the needs of the study. Such a course could provide instruction to all field personnel on the details of standardized data collection and voucher preparation (if relevant), in addition to identification of all species in the target area. On-the-job training in the field can include pairing an experienced person with an inexperienced person, a proven way to bring a new person into the team and ensure that he or she learns the necessary, practical information. Finally, the project leader should perform periodic quality audits, making sure that everyone is taking all the required data properly and consistently. Actual data should also be checked on a regular basis. Frequent evaluations in the lab and field often reveal places where procedures can be modified and improved, ensuring a successful project.

Some or even most field personnel are self-motivated and work well independently. Other assistants, however, especially students, volunteers, or temporary employees, may need more hands-on management to maintain their interest level. Such personnel may not understand the concept and breadth of the entire project or the value of collecting data in specific ways. It is always helpful to provide this information during training. Unless they are given vested interest in the project, such as promised authorship on papers, their motivation levels may vary as the project progresses. Including students or employees as authors on spin-off papers helps to maintain motivation, but acceptable financial remuneration can also maintain interest. Even so, I have witnessed people lose interest as a project nears its end, with a corresponding decline in performance. They are thinking about their next job or, in some cases, graduate school. This is simply human nature, so the challenge is to figure out how to maintain motivation throughout the entire project. It helps if project leaders learn what interests each person on the team and use a reward system that works for them. Most importantly, however, project leaders must hire self-motivated, independent people in the first place, so some skill in recognizing such personalities is required.

Different personality types process information in very different ways. In my experience, so-called "detail people" process and perform differently from "concept (or "big picture") people." Detail people focus on specific aspects of a task and do not necessarily see how the tasks or details fit into the overall concept of the project. Concept people may overlook small but important details of the project but easily envision its full scope and importance. Obviously, not all people fit neatly into one of these groups; rather, most will fall somewhere along a continuum between them. The challenge for project leaders is to recognize such personality traits and learn how to manage people so that everyone works together to complete tasks efficiently, accurately, and on time. The project leader must learn how to work with diverse people having different motivational requirements, experience, work habits, and ways of processing information. All of these factors directly affect the final product.

Using Geographical Information Systems to Design Reptile Surveys

Charles R. Peterson and John R. Lee

Although several investigators have described the use of GIS to measure landscape variables (e.g., Richter and Azous 1995; Bosakowski 1999; Diller and Wallace 1999; Knutson et al. 1999), to design surveys (Hayek and McDiarmid 1994; Fellers 1997; Peterson et al. 2005), and to store and analyze biodiversity data (August et al. 1996) in amphibian studies, relatively little has been written about the use of GIS in reptile surveys. In this paper we show how Geographic Information Systems (GIS) and other geotechnologies (Global Positioning Systems [GPS], remote sensing, and geostatistics) can be used for designing and conducting reptile surveys. We discuss what is possible with GIS, how one gets started, some limitations of the technology, and where to go for additional information. The paper is a modification and extension of an essay describing the use of GIS to design amphibian surveys (Peterson et al. 2005). Examples are drawn primarily from our own experiences using GIS to design reptile surveys in the northern Intermountain West of the United States for a variety of state and federal agencies, corporations, and conservation organizations. We illustrate our approach with a detailed case study of a sampling design for a reptile survey conducted for Craters of the Moon National Monument and Wilderness in Idaho.

The availability and utility of geotechnologies have increased greatly within the past 20 years (for general descriptions of GIS, see Clarke 1997, Heywood et al. 1998, and Krzysik 1998). In the past, GIS required expensive workstations, difficult-to-use software, expert technicians, and considerable resources for acquiring spatial data in a digital format. Now, systems using relatively inexpensive computers and programs (e.g., ArcView, Environmental Systems Research Institute [ESRI] 1999; see Appendix II) have the ability to perform most GIS functions. The software is easier to use, and training is widely available. Courses are offered at colleges and universities, commercial workshops, and online (e.g., via the ESRI Virtual Campus; see Appendix II). Global Positioning Systems (Leick 1995; Letham 2001) allow investigators to find preselected study sites and to map the locations of observations, trapping sites, survey areas, and habitat features. Remote sensing (Berlin and Avery 2003) and digital image processing (Jensen 1996) have provided large amounts of spatial data in digital formats. Digitized aerial photographs, satellite imagery, and other types of spatial data (e.g., topographic and hydrologic maps) now can be obtained on a CD-ROM and over the Internet (see Table 5). A promising development in the use of geotechnologies is the increasing application of geostatistical procedures to environmental studies (Cressie 1993; Bailey and Gatrell 1995; Johnston et al. 2001). Such procedures effectively incorporate data on spatial relationships of environmental variables and animal occurrences and distributions (Scott et al. 2002). The role of geotechnologies in designing animal surveys, conducting fieldwork, analyzing survey results, and sharing information will undoubtedly continue to increase. Indeed, for many survey projects, it is now expected that the results will be provided in a format that can be readily imported and incorporated into the sponsoring organization's GIS.

Role of Geotechnologies

Geotechnologies can contribute to the design of reptile surveys in several ways. A GIS can give biologists a better understanding of the landscapes in which they will be working (topography, geology, hydrology, climate, vegetation, roads, etc.). It can facilitate the organization and visualization of preexisting reptile data, thus helping to identify data gaps. Most importantly, if the appropriate data are available, a GIS can help identify and classify habitats and select sampling sites in an efficient and less biased way through the implementation of systematic, random, stratified, and/or other sampling approaches. It also can incorporate a variety of constraints (e.g., site inaccessibility) into the process of selecting study sites. GPS makes it relatively easy to navigate to preselected sampling sites in the field and to record the spatial coordinates of observations (e.g., den sites) and important habitat features. GIS can also play an important role after surveys are completed by displaying and analyzing results, predicting occurrences in areas not sampled, and modeling the effects of habitat change or management actions on animal distributions and numbers.

TABLE 5

Selected Internet GIS Data Sources and the Data Available[a, b]

Data Source	*Available Data*	*URL*
U.S. Geological Survey (http://www.usgs.gov/)	DRG[c] Topos: 1:250K, 1:100K, 1:63K, 1:20–30K	http://nationalmap.gov/ustopo/index.html
	DLG[d]: 1:2000K, 1:100K, 1:20–25K	http://eros.usgs.gov/#/Guides/dlg
	DOQ[e]: Variable availability. 1–2 m resolution	http://online.wr.usgs.gov/ngpo/doq/
	DEM[f]: 2-Arc seconds, 1-degree, 1:20–25k (10- or 30-m resolution)	http://rmmcweb.cr.usgs.gov/elevation/
	Geology and Mineral Resources	http://mrdata.usgs.gov/index.html
	Hydrography: Hydrological Unit Codes, Rivers, Streams, Lakes, Springs, Wells	http://water.usgs.gov/GIS/
	Land Cover. Resolution: 30 m	http://landcover.usgs.gov/landcoverdata.php
U.S. Environmental Protection Agency (http://www.epa.gov)	Various data at the State and County Level: Census, TIGER[g] roads, TIGER railways, TIGER political boundaries, TIGER hydrography	Enviromapper for water/watersheds http://map24.epa.gov/emr/
National Wetland Inventory Maps	Digital Data available for 39% of Conterminous U.S. Resolution: 1/4 to 5 acres.	http://wetlands.fws.gov/
National Gap Analysis Program	State Land Cover, Predicted species distribution	http://www.gap.uidaho.edu/
National Geophysical Data Center	Various: Satellite Imagery, Glaciology, Climate, Topography, Geothermal, Habitat, and Ecosystems	http://www.ngdc.noaa.gov
PRISM Climate Mapping	Precipitation, Humidity, Temperature	http://www.ocs.orst.edu/prism/prism_new.html
GIS Data Depot	DRG Topos, DOQQ[h], DEM, Hydrography, NWI[i] Land use/Land cover, political boundaries	http://www.gisdatadepot.com/
ESRI ArcData Online	DRG Topos, Aerial Photos (limited availability)	http://www.esri.com/data/online/index.html
All Topo Maps	DRG Topos: Limited (15 States)	http://www.igage.com/
National Spatial Data Infrastructure	Geospatial data Clearinghouse	http://www.fgdc.gov/nsdi/nsdi.html
Rocky Mountain Mapping Center	More links	http://rockyweb.cr.usgs.gov/

[a] Because Internet addresses may change, we suggest using search engines to locate sites for specific data required (e.g., “GIS data,” “Geographic Information Systems data,” “spatial data,” “Digital Raster Graphics,” etc.).

[b] Other data and tools available on the Web include National Wetland Inventory classification maps, vegetation and/or land cover data and maps, Satellite Imagery (Landsat, SPOT, etc.), climate and weather (e.g., temperature, precipitation), and soil.

[c] DRG = Digital Raster Graphics.

[d] DLG = Digital Line Graphics.

[e] DOQ = Digital Orthophoto Quadrangle.

[f] DEM = Digital Elevation Model.

[g] TIGER = Topologically Integrated Geographic Encoding and Referencing.

[h] DOQQ = Digital Orthophoto Quarter Quadrangle.

[i] NWI = National Wetland Inventory.

VISUALIZING STUDY AREAS AND DATA AVAILABILITY

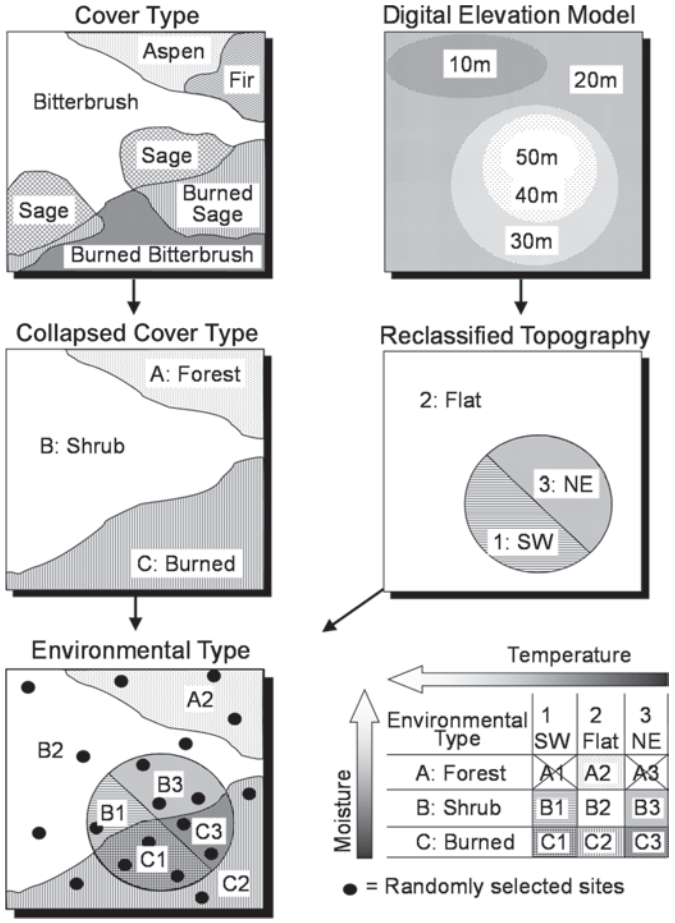
The increasing availability of GIS data has greatly facilitated the ability of investigators to develop an appreciation for the characteristics of a study area (Table 5). U.S. Geological Survey (USGS) topographic maps, aerial photographs, National Wetland Inventory (NWI) maps (Cowardin et al. 1979), and similar spatial data have been available in nondigital format for a number of years. After being digitized, this information can be layered in a GIS with other data, providing a more complete and integrated picture of the landscape in a study area and, therefore, a better understanding of it. For example, DOQQ (digital ortho quarter quad) aerial photos are useful for identifying topographic features and cover types (e.g., water, barren areas, patches of vegetation). For some areas, color DOQQs are available, making it easier to identify certain habitat features (e.g., wetlands). The availability of other types of data, such as vegetation type, soil type, precipitation, and temperature, in GIS form makes it more feasible to incorporate these variables into a survey design. Until recently, detailed GIS data were limited, but now a wide variety of spatial data are available (Table 5), and organizations such as national parks or utility companies commonly have extensive GIS databases. Existing data can be used to derive data layers (e.g., slope and aspect from Digital Elevation Models [DEMs]). Spatial relationships, such as the isolation of a den site from other dens or the distance of sites to roads, also can be determined with GIS.

Because reptiles generally use their environments at a relatively fine spatial scale (see "Describing Microhabitats," in Chapter 4), identifying small habitat features, such as rocks, cave openings, vegetation patches, and ephemeral wetlands, is often important. Distinguishing such habitat features often requires data with both high spatial resolution (<5 m) and high spectral resolution (many narrow radiation bands vs. a few broad bands). Although such data are becoming increasingly available, the lack of data appropriate for identifying and classifying reptile habitat is still a major problem in many areas. Data obtained with relatively new types of remotely sensing, such as *laser altimetry,* or *Lidar* (*light detection and ranging*; Lefsky et al. 2002), and hyperspectral imagery (Richards and Jia 1999) should prove useful for generating detailed habitat maps. Laser altimetry can be used to create fine (e.g., 20-cm vertical resolution) topographic maps on which many habitat features (e.g., talus slopes and boulders) can be identified and vegetation height, cover, and canopy structure can be measured (Lefsky et al. 2002). Results from a preliminary study using high spatial resolution and hyperspectral imagery to map amphibian habitat in Yellowstone National Park are also promising. Using 5-m resolution hyperspectral images obtained with a PROBE 1 sensor (128 spectral bands) on a fixed-wing aircraft, we were able to generate relatively accurate (ca. 85% correct classification of 32 sites) maps of habitats (sedge wetlands and pools with algae) predicted to be appropriate for amphibia (C. R. Peterson and W. A. Marcus, unpubl. data). We located several new anuran sites that we probably would not have identified if we had only been using conventional techniques (e.g., topographic and NWI maps). Hyperspectral data should also prove helpful for mapping reptile habitats (e.g., based on geology, soils, and vegetation) once they are more commonly available and analytical techniques are better developed.

A GIS facilitates the organization and visualization of preexisting data and, thus, helps to identify data gaps. One of the first steps in planning a reptile survey is to determine the species of reptiles likely to occur within the study area. This helps to ensure that the appropriate sampling techniques are selected so that no species is missed. Typically, a list of potential species is compiled from field guides, published literature, and museum collections. Other sources of information include Nature Serve (http://www.natureserve.org), state Gap Analysis projects (http://www.gap.uidaho.edu/), and state or regional herpetological databases. To be incorporated into a GIS, these data must be in tabular form with localities expressed as digital coordinates (e.g., decimal degrees or Universal Transverse Mercator coordinates). Increasingly, coordinates measured with a GPS receiver are being used to report locations. Since May of 2000, when the military discontinued the intentional degradation of GPS signals, accuracies of better than 20 m have been obtained with even relatively inexpensive GPS receivers. For older observations and museum specimen records, however, we typically use the written descriptions to find locations on digital topographic maps (Digital Raster Graphics [DRGs]) on a computer monitor. The use of a geographic feature index (e.g., USGS Geographic Names Information System [GNIS]) greatly facilitates finding a feature on a map and reduces the likelihood of confusing different features with the same names (e.g., 30 of 44 counties in Idaho have at least one stream named Spring Creek!). We assign each recorded location an accuracy rating (in meters) that represents the radius of a circle around the digitized point in which we are highly confident that the actual specimen location falls. These locality data can be used to generate point distribution maps for individual species. If reptile surveys have been conducted in the area previously, plotting the locations of all survey sites where appropriate collecting techniques were used can help determine if the lack of a record for a particular species in a particular area is due to species rarity or to insufficient survey effort (i.e., data gaps). Including all locations where reptiles of similar "detectability" have been observed also helps to address this question.

SURVEY OBJECTIVES, SAMPLING SCHEMES, AND GIS

How a GIS is used to select sampling sites is likely to vary with the objectives of the survey (e.g., to determine occurrence, distribution, abundance, habitat relationships, or population status/trends). For simple occurrence within a study area such as a national park, using a GIS to focus first on those habitats where reptiles are most likely to be found (e.g., snake denning sites) probably is the most appropriate approach. Selecting sampling sites with a systematic scheme may be the best approach to determine the spatial distribution of reptiles within the study area empirically. If a geostatistical technique like *kriging* (a statistical interpolation technique that uses data of a single type to predict values of that data type at unsampled locations; Johnston et al. 2001) is used, sampling sites at different intersite distances should be selected. Measuring abundance may be improved by incorporating adaptive sampling procedures in which the results of the sampling at an initial set of sites determines the placement of subsequent sites (Thompson and Seber 1996). A study of habitat relationships should minimize bias and include sites where the species does and does not occur, usually requiring some random component to the site selection. Including sites with historical data would be appropriate for a survey attempting to determine species status and trends in the study area. Complete spatial surveys are apt to be needed to evaluate the probability of reptiles persisting



Environmental Type	1 SW	2 Flat	3 NE
A: Forest	~~A1~~	A2	~~A3~~
B: Shrub	B1	B2	B3
C: Burned	C1	C2	C3

FIGURE 4 Stratification and randomization of sampling-site locations with a GIS. In this example, six original cover types are collapsed into three types to reflect moisture availability in the habitat. Slope and aspect are combined and reclassified into three topographic classes to reflect surface temperature. The environmental types are created by intersecting these two coverages. Note that of the nine environmental types possible, only seven actually exist in this example.

within a study area (see Pilliod and Peterson 2000 for an amphibian example). More than one sampling design will likely be required to achieve multiple objectives.

STRATIFICATION AND RANDOMIZATION IN SITE SELECTION

Stratifying and selecting sampling sites in ways that are efficient and minimize bias are potentially the most important uses of a GIS when designing reptile surveys. Using systematic, random, and/or stratified sampling approaches to select sampling sites is described in this volume (see “Random Sampling: Unrestricted and Restricted,” under “Some Precautions for Quantitative Reptile Field Studies,” below) and in Hayek and McDiarmid (1994), Fellers (1997), and Thompson et al. (1998). Systematic schemes such as grids or transects can be imposed on a study area. While random selection of sampling areas or sites does not require previous knowledge of reptile habitat relationships, adequately describing the range, distribution, and conditions associated with occurrence of low-density species may require large numbers of samples. Options for randomly selecting sites within GIS thematic layers exist in programs such as ArcInfo and the Animal Movement Extension (Hooge and Eichenlaub1997) for ArcView. Factors that may explain variation in the dependent variables of interest can be used for stratifying the sampling sites. These factors can be physical (e.g., temperature or moisture availability), biological (e.g., predator or prey abundances), or spatial (e.g., patch size, distance from an edge/feature). If the required habitat information is already in the GIS, the sampling scheme can incorporate the range of variation in those factors, but it does not need to determine their actual distributions.

Gradsect sampling (Austin and Heyligers 1991), in which transects are placed so that the steepest gradients in variation for the desired factors are sampled over the smallest spatial area, can do this efficiently. Multiple factors may be incorporated into the stratification. For example, three cover-type classes combined with three topographic classes would define nine environmental types for allocation of sampling effort (Fig. 4). In the GIS, such a combination may be done manually by simply intersecting and reclassifying the appropriate coverages. For more complex schemes, such as when the coverages representing the desired factors need to be derived from multiple coverages, tools such as ArcView’s model builder extension (ESRI; see Appendix II) can be of great use.

INCORPORATING CONSTRAINTS

GIS can also be very useful for incorporating a variety of constraints into the selection of sampling sites. If a study area is small enough or if sufficient resources are available, constraints are less of an issue and a complete survey (census) of all habitats is possible. Unfortunately, most studies are faced with numerous constraints, making it necessary to subsample within a study area. GIS can assist by incorporating many of these constraints into a design a priori. These constraints can arise from several sources, including limited resources, the topography of the study area, road access, environmental or historical sensitivity (i.e., rare/threatened species, archeological sites, etc.), and other uses (e.g., tourism). For example, to avoid spending excessive time traveling to sites, researchers may limit sampling to areas within 5 km of a road or trail. In Yellowstone National Park, conducting surveys in grizzly bear management areas is not allowed during certain times of the year. National parks may also restrict sampling to areas out of public view. GIS can be used to help find such areas by producing viewsheds, or polygon maps resulting from a visibility analysis that show all locations visible from a specified point (Heywood et al. 1998). Viewshed analysis can also be used to select trapping sites that are less likely to be disturbed by vandals.

SITE SELECTION AND VALIDATION

The potential for error inherent to GIS databases and their manipulation necessitate ground truthing potential sampling sites before the sites are finally selected. Inaccuracies within the initial coverages include positional errors (incorrect *xy*-coordinates for points, lines, or polygons), attribute errors (incorrect or missing descriptive data), or a combination of the two (DeMers 2000). Such errors propagate unpredictably when multiple coverages are combined into the final environmental stratification. Because these errors are inherent in most spatial data sets, investigators usually generate more points per environmental type than necessary for sampling and then ground truth each point. Actual sampling sites are selected from the potential sites accurately classified by the GIS. Verification of the characteristics at each point may involve measuring the attributes assigned by the GIS. Even some of the lower-priced (~$200) GIS receivers currently available can display the boundaries of an area to be ground truthed and an observer's position relative to or within the area. More expensive models (Trimble Geoexplorer CE, ~$5,000) can display such information on digital photos (DRG, DOQQS, or DLG [Digital Line Graphs]) or other map coverages on the screen in the field.

ACCURACY ASSESSMENT

To generate the best possible estimate of coverage accuracy, the area ground truthed should match the resolution of the respective coverages (which may entail measuring different attributes over different areas). Site verification ensures that the correct environmental type is being sampled; it can also generate accuracy estimates for the initial and stratification coverages. In many cases, accuracy of the initial coverages may not be available a priori, and estimating the accuracy of the stratification coverage may be necessary for evaluating the results of the final analyses. For example, many researchers would be quite satisfied if their study produced a statistical model predicting probability of occurrence for a species with a classification accuracy of 95 percent. However, if this model were combined with a stratification coverage that was only 75 percent accurate, the resulting predicted distribution map most likely would be less than 75 percent accurate. Unfortunately, the exact manner in which these errors are propagated is unpredictable, especially if the coverages used are from different sources (Chrisman 1987).

Additional Uses for GIS in Reptile Surveys

GIS can also be employed after the field data are gathered. Investigators can use GIS to visualize and analyze the results of a survey (e.g., view distributional patterns, determine distances from survey sites to particular environmental features, calculate landscape variables, and assess habitat associations), for predicting occurrence in areas not sampled, and for modeling the effects of habitat change and management activities. Researchers can also use GIS to communicate information effectively to managers and policy makers (e.g., as maps to supplement oral presentations, or graphics in a report; Peterson 1997). GIS data can be integrated into the information systems of organizations so that data will be more readily available when they are needed for planning purposes. Spatial data and maps also can be distributed to the public over the Internet with programs such as ArcIMS (ESRI, see Appendix II).

Case Study: Craters of the Moon

In this section, we describe how we used a GIS to develop and implement a survey of terrestrial reptiles at Craters of the Moon National Monument and Wilderness Area near Arco, Idaho. The objectives of the survey were to determine the distributions, relative abundances, and species–habitat associations of the reptiles present. Our first step was to use a GIS to stratify the study area into environmental types based on the factors (temperature and moisture availability) that we expected to have the greatest influence on local patterns of reptile distribution and abundance. We generated sets of randomly selected potential sites within each environmental type and then ground truthed each potential site, collecting habitat data for accuracy assessment of the GIS classifications. From the correctly classified sites, we selected the actual sampling locations. At each sampling location, we installed drift fences with funnel traps (see "Funnel Traps, Pitfall Traps, and Drift Fences," in Chapter 5, and "Pitfall-Trap Surveys," in Chapter 13) to capture and document the species of reptiles occurring in the different environmental types. This GIS-based approach, detailed below and in Figure 5, allowed us to construct spatially explicit models for the probability of occurrence for each species across the entire Monument and to incorporate our results as baseline data into a protocol for future monitoring.

For the initial stratification, we used ArcView version 3.2 to generate a map of environmental types. We used moisture and heat availability to define the environmental types, but these two properties cannot yet be directly mapped, so we used other already existing information as surrogates. For moisture availability, we used the Monument's cover-type map (Day and Wright 1985). Because of logistical constraints, we collapsed

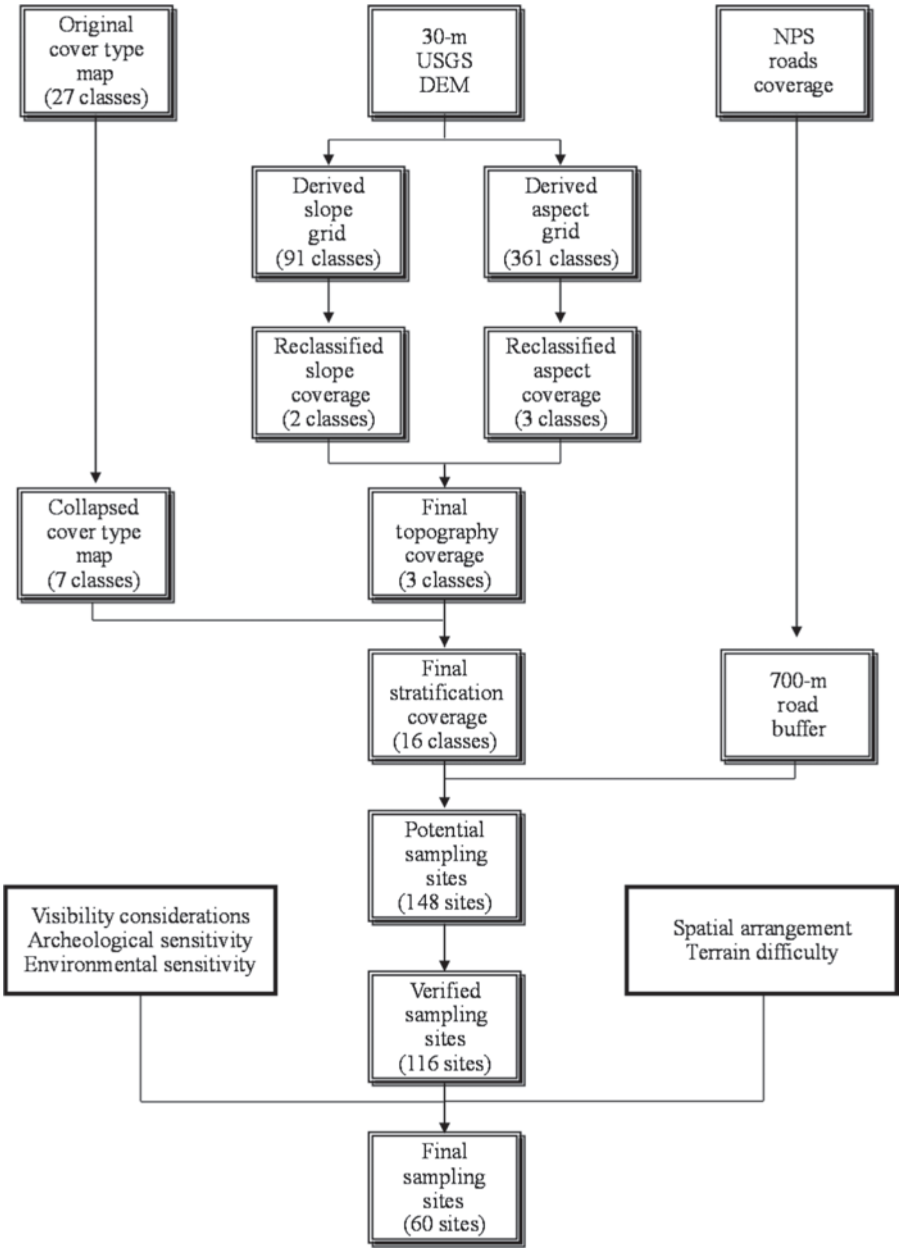

Original cover type map (27 classes)
30-m USGS DEM
NPS roads coverage
Derived slope grid (91 classes)
Derived aspect grid (361 classes)
Reclassified slope coverage (2 classes)
Reclassified aspect coverage (3 classes)
Collapsed cover type map (7 classes)
Final topography coverage (3 classes)
Final stratification coverage (16 classes)
700-m road buffer
Potential sampling sites (148 sites)
Visibility considerations
Archeological sensitivity
Environmental sensitivity
Spatial arrangement
Terrain difficulty
Verified sampling sites (116 sites)
Final sampling sites (60 sites)

FIGURE 5 Flowchart demonstrating how GIS was used to stratify and select sampling sites for reptiles at Craters of the Moon National Monument and Wilderness. (DEM = Digital Elevational Model; NPS = National Park Service; USGS = U.S. Geological Survey)

the 27 defined cover types into seven classes such that the moisture requirements of the vegetation within each class were more similar than the moisture requirements of the vegetation between classes. To represent differences in relative surface temperature, we defined three topographic classes based on the differences in the amount and timing of incident solar radiation due to slope and aspect. We used 30-m USGS DEMs to generate slope and aspect information, reclassifying it before combining it into our topographic classes. The 91 classes (representing slopes of 0 – 90°) of slope information were collapsed into 2 classes: flat (slope ≤ 5°) and hill (slope > 5°). The aspect information was reclassified from 361 classes (1– 360° plus one class for no aspect) to 4 classes: no aspect, flat areas, NE (135–315° aspect), and SW (135–315° aspect). We intersected the reclassified slope and aspect coverages into our final 3 topographic classes. The final classes were as follows: flat (areas with ≤5° slope and any aspect), NE slope (areas with >5° slope and an aspect between 315 and 135°), and SW slope (areas with >5° slope and an aspect between 135 and 315°). Intersecting the collapsed cover-type coverage with the topography coverage generated the environmental-type stratification coverage (Fig. 6). Of the 21 potential environmental types (7 cover types × 3 topography classes), only 16 actually existed in the Monument (Table 6). Using the X-Tools extension (DeLaune 2000), we calculated the area of the individual environmental-type polygons and allocated sampling efforts roughly proportionally to the total area of each type (Table 6). We made sure over the 2.5 years of the study that the rare environmental types had at

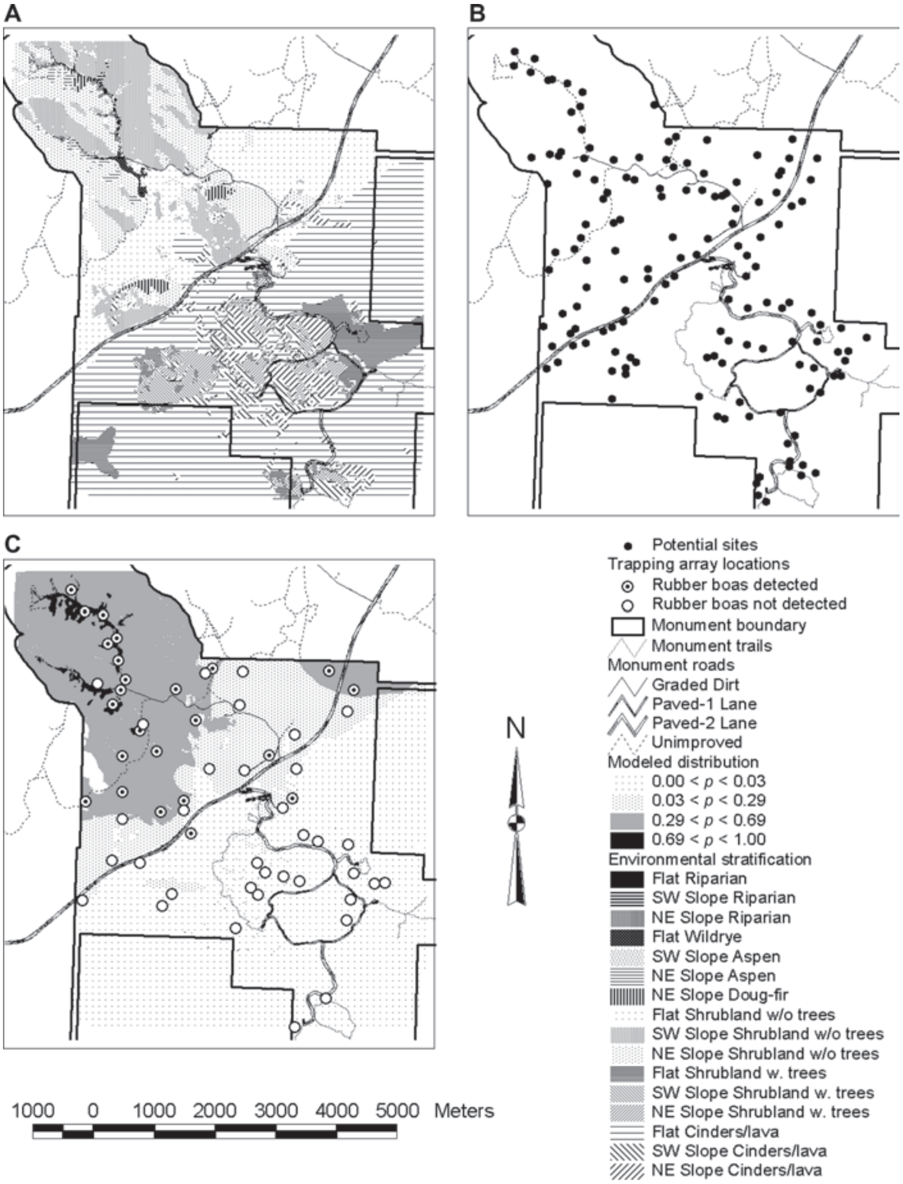


FIGURE 6 Case study for Northern Rubber Boa (*Charina bottae*) at Craters of the Moon National Monument and Wilderness. (A) Environmental stratification for the study area. (B) One hundred and forty-eight randomly generated potential sampling sites within the environmental types. (C) Trapping results and predicted probability of occurrence map for rubber boas. This predicted distribution has an overall classification accuracy of 89.9 percent when a threshold probability for occurrence of $p = 0.29$ is used.

least one replicate and that the most common environmental types had two to three replicates each. Some types were sufficiently rare that subsampling was unnecessary; one or two sampling sites effectively provided a census of the type.

Within each environmental type, we generated sets of randomly selected potential sites that were ground-truthed for classification accuracy. Using the Animal Movement Analyst extension (Hooge and Eichenlaub 1997) to ArcView, we randomly generated *xy*-coordinates for potential sites within the polygons for each environmental type. The effort required to check trapping arrays caused us to limit selection of the initial sampling sites to relatively accessible areas. We did this by generating a 700-m buffer around the roads in the transportation coverage for the Monument and excluding points falling outside the buffered areas. We generated three to five times more points (total = 148) for each environmental type than we actually planned to use (Fig. 6B). Field survey teams used GPS units to navigate to all of the potential sites for ground truthing. The teams collected data on slope, aspect, substrate, and vegetation for the 30- × 30-m area surrounding each point and took voucher photographs of each site. Because we would be trapping in areas of the Monument visited by the public, we also assessed if a drift fence placed at each site would be visible to visitors.

From the sites that had been accurately classified by the GIS stratification, we selected the actual sampling locations based on criteria for effort, spatial distribution, and visibility. To conserve effort, we selected clusters of sampling sites when points in different environmental types fell within a single area having a 1,000-m radius. To maximize spatial distribution, we se-

TABLE 6

Stratification[a] and Effort Allocation[b] for a Reptile Survey at Craters of the Moon National Monument and Wilderness Area, Idaho

Environment Covertype	Topography Southwest	Topography Flat	Topography Northeast	Total
Area (ha) of each environmental cover type				
Wild rye	0	11	0	11
Riparian	6	5	11	22
Aspen	2	0	20	22
Douglas Fir	0	0	27	27
Shrubland with trees	69	218	110	387
Shrubland without trees	404	563	483	1450
Cinders/lava	125	1606	171	1902
Total	596	2403	822	3821
Trapping arrays allocated to each environmental type				
Wild rye	n/a	3	n/a	3
Riparian	2	1	0	3
Aspen	1	n/a	2	3
Douglas Fir	n/a	n/a	3	3
Shrubland with trees	4	8	6	18
Shrubland without trees	7	10	5	22
Cinders/lava	3	3	2	8
Total	17	25	18	60

a. Stratification: area of each environmental cover type.

b. Effort allocation: trapping arrays allocated to each environmental type.

lected the clusters such that distances among clusters were maximized. At each sampling location, we installed drift fences to capture and document the species of reptiles occurring in those environmental types. The drift fences were open during three consecutive field seasons from 1999 to 2001. As an example, the capture results for Rubber Boas (*Charina bottae*) are shown in Figure 6C. Rubber Boas were detected at 24 of the 60 sampling locations; using those results, we constructed the predictive distribution model shown in Figure 6C. The statistical model was generated through logistic regression analysis of the capture data. The logistic regression model incorporated the stratification information by predicting probability of occurrence from ranked moisture availability and topographic class. We then applied this model to the environmental stratification map shown in Figure 6A by calculating the probability of occurrence for each polygon based on its attribute values for cover type and topographic class. To make use of the spatial autocorrelation of the dataset, we performed indicator kriging (Isaaks and Srivastava 1989) on the dataset at well. By intersecting the kriged coverage with the environmental stratification coverage, we weighted the regression probabilities by multiplying them by the kriged probabilities. This had the desired effect of "trimming" the habitat-based regression model down to the spatial area over which the species was actually detected. When a cutoff threshold value of $p > 0.29$ (as derived from analysis of the ROC plot; Swets 1988) is used, this model has an overall classification accuracy of 89.8 percent. This GIS-based approach for constructing spatially explicit models for the probability of occurrence allows us to present resource managers with actual maps of where each species is expected to occur. These maps can be combined to produce species-richness maps for the study area and used to predict the effects of a particular habitat change on the organisms.

Acknowledgments

Stephen Burton, Stephen Corn, Ted Koch, Debra Patla, and David Pilliod helped to develop the ideas presented here. John Apel, from Craters of the Moon National Monument, provided invaluable assistance. Yellowstone Ecosystems Studies generously supported the hyperspectral analysis study.

Precautions for Quantitative Reptile Field Studies

Lee-Ann C. Hayek

Because of the frequency with which one hears about "lies, damned lies, and then statistics," statistics has become a pejorative word despite representing a reputable scientific field of mathematical concentration. Consequently, there is a wide-ranging need to address the aspects of statistical thought that have led to such distrust as well as the misuse and misinterpretation of statistical findings. I attempt to do so here, specifically for the community of reptile biologists. I have organized this section into a triad of problem areas for both observation and analysis: (1) data collection and sampling, (2) common methodological errors, and (3) misinterpretation of results and misapplication of techniques. For each I present precautions that should be taken by reptile researchers who intend to quantify their field results and subject their observational data to statistical description and inferential assessment. The comments herein are not sufficient to allow an investigator to solve all of his or her statistical problems, nor are they all-inclusive. Investigators must still conform to statistical theory. And although acknowledging problems and taking precautions to avoid them are beneficial to any project, they will not make the reader a mathematical statistician. I recommend, therefore, that investigators seek advice from a mathematical statistician during the design stage of their research and throughout the data analysis and interpretation stages, a practice that is vital to the success of field studies.

Field Study Design Problems

Statistics can be descriptive or inferential. The function of *descriptive statistics* is to express observations in a way that allows us to learn from them. For field data to provide usable information on the reptiles of interest, we must do the following:

1. Control the variability of factors within our study design.
2. Summarize field results to emphasize communal properties of the population of interest and to subsume individual differences.
3. Look for and interpret patterns among the results of our observations.

Inference, in contrast, allow us to draw conclusions that extend beyond our immediate data set. The purpose of *inferential statistical methods* is to allow us to infer something about a target population of interest based on information obtained by sampling individuals or groups of reptiles. Of interest is what observed data can tell us about a larger set of observations; a wider or more complex sample area, group, or assemblage; or the population from which the initial sample was taken. To make valid inferences from sampled field data, we must adhere to set mathematical statistical principles and ensure that the assumptions we make about the population and its related data are valid.

PROBABILITY, STATISTICS AND UNCERTAINTY

In colloquial terms, uncertainty about any aspect of an issue induces doubt or insecurity about how to deal with or interpret that issue. Uncertainty can arise from ignorance, from ambiguity in the nature or context of an issue, or from an inherent unpredictability of the situation. Probability is a mathematical statistical theory developed both to describe and to quantify uncertainty. Quantification of uncertainty by means of the theory of probability tends to remove doubt or at the least to minimize misgivings. Statistics, especially when applied to large data sets, can relieve the anxiety induced by uncertainty in data.

The field of statistics was developed as a means of organizing and summarizing large amounts of data collected for government purposes. While statistics still deals with the collecting and summarizing of data, it now approaches the task differently. Modern statistical science uses mathematical probability theory, first to describe and summarize or model uncertain events, and then to analyze and interpret observed data. Both our knowledge and our ignorance of natural reptile populations that have been sampled can be described mathematically. Uncertainty about observed events is measured on a scale of 0 (impossible, not observed) to 1 (certain) and is expressed as a frequency or probability and then constrained according to a developed theory or model. *Constraints* are bounds or limits on data statements; their use increases confidence in both our observed results and our interpretation of them. The key to having confidence in results is to rely upon the rules of statistical science.

Validity, which is measured against external criteria, is the extent to which any procedure or tool evaluates the variable that it was designed to evaluate. That is, valid procedures adhere to their stated purpose(s). Statistical validity is the extent to which an indicator (test, instrument, measure, estimate, or other) measures what it was devised to measure. Validation of procedures against independent or external measures (e.g., ground truthing data obtained from remote sensing) can indicate the degree to which those procedures are either free of bias or minimize it. Investigators validate a sample or field investigation statistically in an attempt to show that the data collected are accurate and representative of the population. Below I discuss ways to improve the validity of investigations so that an investigator can be confident that his or her study design and fieldwork will provide a valid test of a hypothesis.

ASSUMPTION OF RANDOMNESS

Variability in a field study can be so extensive as to be a nightmare. However, investigators can control such variability with an appropriate study design based on statistical considerations. A critically important assumption for most standard statistical tests and formulae is that observations or other measurements are collected from a randomly chosen sample of the population. The validity of a statistical result or conclusion is connected directly to the validity of the randomness statement, as well as of other assumptions.

Knowing or being able to assume the pattern or limits of variability for a specific population increases a researcher's ability to avoid measurement error and to validate procedures. It is vital to understand that in a field study, randomness is a characteristic of the process by which data are gathered, not of the data themselves. Probability statements, parameter estimates, and test results obtained from any statistical analysis may be incorrect and invalid if data collection is not random. It is widely known that many statistical tests are "*robust*" to their assumptions, that is, that the test results are little affected by the data's departure from the strict adherence to the assumptions. However, the property of robustness is never a license to ignore an assumption, especially one as fundamental as randomness. We can relax an assumption or loosen a prerequisite if there is mathematical statistical proof that results will remain valid. Nevertheless, a person designing a study must not ignore the responsibility of adherence to assumptions.

RANDOM SAMPLING: UNRESTRICTED AND RESTRICTED

Most researchers who use statistics realize that an ideal field sample is one drawn in circumstances under which each member of the population can be selected at random. In such a sample, the randomness assumption clearly obtains. Unfortunately, fieldwork on natural populations of reptiles or most other organisms is not likely to occur under such circumstances. Possibly the most important aspect of random sampling for fieldwork is a clear understanding of the distinction between a biological sample and a statistical sample. For an investigator surveying reptiles, for example, the return from a single litter plot or transect is a biological sample. However, statisticians generally call each of these units an "observation" rather than a "sample," and what is called the statistical sample is, in contrast, the set of all observations, or all biological samples. Thus, *simple (unrestricted) random sampling* in fieldwork means that each possible combination of n biological samples has an identical chance of becoming the observed statistical sample of size n, or, in other words, that each biological sample has an equal chance for selection. Since this is true, then clearly simple random sampling does not necessarily mean that each individual organism in a population must have an equal selection probability, or chance of being selected. For example, in a study (e.g., of some types of density estimation) in which the biological sample is a quadrat, each quadrat, not each individual, must have an equal probability of being selected. When each biological sample, be it individual organism, quadrat, or transect, has an equal selection probability, sampling is unrestricted and random.

Under some circumstances, unrestricted random sampling is neither the simplest nor the most efficient method for inventorying or monitoring reptiles in the field. For all fieldwork with natural populations (Hayek and Buzas 2010; Hayek and Heyer 2003), it is sometimes profitable to restrict simple random sampling. *Restricted random sampling* is sampling upon which conditions have been imposed but that still provides data sets that are suitable for statistical description and analysis. *Stratified sampling* is one type of restricted sampling. For example, when a study design calls for stratification (stratified sampling), the reptile population of interest (i.e., the target population) is divided into parts called strata, for consideration. Within each stratum, which can correspond to a habitat, a subpopulation (e.g., males or females, juveniles or adults, etc.), or a gradient, the sample is selected at random. Hayek and Buzas (2010) discussed study design and the interrelationships among each of the restricted methods as well as the advantages and disadvantages of the use of each in the field. Most often when a restricted random sampling procedure is used correctly, the resultant sample estimates are more precise than those estimates from unrestricted random sampling.

Each restricted method depends for its increased precision upon two factors: (1) that the samples are spread over the population to minimize the possibility of samples that are highly unusual, and (2) that sampling intensity can be increased (a larger sample taken) in areas most useful for achieving the study objective. For most reptilian populations, stratification with replicates taken in each stratum, or replicated systematic sampling, will be quite useful. With both sampling designs (restricted and unrestricted), the investigator obtains information on the separate sample units (e.g., habitats, sexes, age groups) as well as on the overall population, because the randomness assumption is valid within each grouping. In 2003 Hayek and Heyer introduced an unbiased statistical sampling strategy, called *Adaptive Cluster Sampling* (ACS), into the herpetological literature. An ACS design, when used with quadrat sampling, overcomes many of the problems associated with field sampling for rare, elusive, or sparsely distributed organisms as well as organisms with nonhomogeneous (patchy) distributions. In such cases, a single, primary set of quadrats is set out, and the reptiles present are determined. If the results satisfy some predetermined condition (i.e., defined prior to initiating sampling) that triggers adaptive sampling (e.g., some minimum number of individuals of the target species is found), then additional contiguously placed quadrats are also examined and the contents are included in the total sample. ACS designs allow all relevant target reptiles or their signs that are located near the initial sample plots to be included in the total sample. Normally, sampling additional quadrats in the vicinity of the original ones based on the presence of target animals would violate principles of random sampling and result in biased estimates. However, the ACS formulae allow one to correct for this problem and obtain an accurate statistical estimate of population size. ACS can be used with either unrestricted or restricted sampling methodologies (Thompson 1991). It provides efficient, small-variance, unbiased statistical estimates of species richness or density of reptiles. ACS can also be used for noninvasive sampling in which the target reptiles are not disturbed prior to counting or attempted capture.

REPRESENTATIVE SAMPLING

Statistical analyses can be used both to describe data and to draw inferences from them. One of the fundamental tenets of statistical analysis when used for inferential purposes is that the field sample is representative of the target population. The purpose of a sampling design that invokes randomness is to provide for the collection of a sample that is, indeed, representative. That is, inferential validity rests both upon the assumption that the observed data (i.e., the statistical sample) is representative of (comparable to) the target population in all characteristics of importance to the study, and the extent to which it is representative. Unfortunately, it is often difficult to obtain a representative sample in the field. For example, a small sample of Xenodontid snakes taken in the neotropics would have a very low probability of containing a reasonably complete representation of species within the family, given that many species are rare. Many inventories and studies of biological diversity suffer from the problems of species unevenness and rarity in which the composition of the sample clearly does not reflect the population either within or across biomes or faunal zones.

If a collected sample is not representative of a population, investigators sometimes can control for differences statistically with, for example, an analysis of covariance (ANCOVA) or a reliability-corrected ANCOVA. Application of such methods often is problematical, however, depending on additional assumptions that were not considered in the original study design. It is always best to evaluate relevant conditions (e.g., environment, life histories, life-cycle events, distributional patchiness, etc.) and design a study that incorporates these factors or considers their effects across the random selections. An extraneous yet relevant variable not considered in a study design can invalidate the results of a study.

If the composition of a field sample is comparable to the composition of a target population, the results from an analysis of the data provide a valid basis for scientific inference. This does not imply that one should try to select only “typical,” or exemplar, individuals. *Representative,* when used in a statistical context, is a property of the observed sample, not of the sampling process. That is, we desire our observed sample to have a meaningful relationship to the target population. A sample that has been selected specifically to mirror a population, sacrificing randomness, cannot be termed representative. If only the smallest, largest, or most easily caught individuals are selected, the methodological problem of bias, to be discussed below (see “Bias, Measurement Error, and Biased Statistical Results,” below), is very likely to be introduced.

No magical number of observed or collected individuals will provide everything one needs to know about a target population. The more individuals counted and identified, the “better” (or more representative) will be the estimates of species numbers and relative proportions, yet the need for this must balance the time, effort, and cost of obtaining large samples. In addition, bigger is not always better when collecting field samples; any statistical test will have a significant result if a sample is large enough. Regardless of the size of the sample, the design of any field study must always be focused on obtaining a representative sample.

To see the importance of having representative sample results, consider the spatial distribution of individual reptiles of a species within a study area. Clearly, it is easier to obtain a representative sample of a population with a homogeneous spatial distribution than it is to obtain one for a population with a widely heterogeneous or patchy distribution. In the former situation, representativeness and precision of the sample increase when more individuals are counted, whereas the size of the biological sample (quadrat or area) can be left to the discretion of the investigator. In the latter case of distributional patchiness or heterogeneity, a large number of small, randomly placed quadrats, or biological samples, should be surveyed rather than a few large biological samples (Hayek and Buzas 2010).

REPETITION AND REPLICATION

Because natural populations are inherently variable, variance estimates should not be based upon single biological samples, regardless of how many individuals are included. That is, the largest of quadrats containing even hundreds of reptiles is still only a single statistical sample. Sampling should always be repeated, either at the same time and place, within the same area or device, or over time and/or space, depending upon study purposes. Repeating a work by obtaining more than one biological sample allows for more precise estimates in the face of natural variation. The purpose of the study and the questions being asked determine the conditions under which a study is repeated.

Repetition is repeating; it is the incorporation of repeated statistical sampling or testing at a distinct point in space or time within a research program. Replication of a field experiment or study is repetition carried out at a single place and, to the extent possible, at a single time. Replication increases precision and provides for closer estimation of sampling error. As explained by Hayek and Buzas (2010, p. 121), it is advantageous to include at least four field replicates in any study design. Developing a sampling plan specifically tailored to the characteristics of the organism based on existing knowledge of a target population increases the likelihood of reproducible results upon repetition within the study or replication of the study. For example, using the advice above for sampling from homogeneously versus heterogeneously distributed populations increases reproducibility of the results of the study.

PSEUDORANDOM SAMPLING AND TRUNCATION

Nonrandom sampling is not always an insurmountable roadblock to statistical analysis of data or model fitting. Efficient parameter estimation and powerful hypothesis testing can still be carried out when data have been obtained by some nonrandom method or by restricted random sampling. However, when an investigator unwittingly or knowingly selects or excludes sample units, sampling is no longer "random," and the inherent pattern of the variability of the field observations is unknown. The rules of probability and statistics do not apply to arbitrarily selected data.

Consider an example in which an investigator truncates or limits the "random" sample. Let us say that the study design calls for observation of the entire contents of each quadrat. However, the researcher observes only the reptiles in the interior of the quadrat because those on the perimeter slither away during the observation procedure. This is an example of accidental truncation of the sample, but it can often be accommodated within the analysis.

Sample limitation can also occur after the data are collected, when regression analysis is applied. The logarithm of species abundance or species effort is often plotted against numbers of individuals collected or counted for predictive purposes (see "Truncation," under "Absolute Estimators I," in Chapter 15). Outlier points are revealed and can be eliminated. A wealth of papers in the statistical and applied literature discuss conditions under which particular field observations can legitimately be eliminated from analysis. All of the conditions are predicated on an aberrant data point's being incorrect or invalid in some manner. Frequently, however, overly enthusiastic researchers eliminate observations based upon their position on the plot, rather than on valid considerations related to study variables. Elimination of an extraordinary or unusual observation merely because it is a statistical outlier is a dangerous and escalating trend in quantitative analysis. With measuring or monitoring projects, this practice will result in smaller estimated standard errors and inflated claims of statistical significance. Such *pseudorandom* (or "false random") sampling can always substantially affect *power* (ability to observe a real effect when it occurs) and probability (α level) of making a type-I error when testing a hypothesis.

Excluding observations for arbitrary reasons in the field or eliminating them from the resultant data set under analysis affects estimates and generally shrinks sample variance. This decrease in variance, in turn, increases the rates at which the hypotheses being tested will be rejected. Thus, introducing arbitrariness (e.g., by eliminating a "large," or "unusual," individual and including a "typical" individual) into a standardized field survey design increases the probability of obtaining a statistically significant result when, indeed, there is none. Eliminating outlier observations also increases the power of a test, a result that can be advantageous if the reason(s) for truncating a data set, either in the field or during the analysis, is legitimate. Compelling reasons for eliminating a data point include such things as instrument error and certain field conditions that cause aberrant observations. The price for an illegitimate increase in power is that the observed probability value of the test will exceed (or even significantly exceed) the actual or true value (the test level). Under such circumstances, it is unlikely that the study will be reproducible or that a finding of significance will survive scrutiny. Sound statistical principles should guide data elimination, and clear justification for removal of observations should be cited, or the resultant analyses, tests, and parameter estimation results are virtually guaranteed to be incorrect.

Methodological Errors in Fieldwork and Analysis

For the study of reptile populations in the field, the core of statistical methodology is its ability to provide a firm foundation for making inferential statements about a target population based upon the field sample. Statistical science provides infrastructure and benefits when the researcher adheres to the statistical and probabilistic guidelines, thereby avoiding methodological error. For statistics to provide such advantages, the researcher must obtain a representative sample whose observed variables conform to assumptions that underlie the statistical process and tests.

Bias, Measurement Error, and Biased Statistical Results

Statistical science aids the reptile researcher both by imparting advice on study designs for inventorying or monitoring reptiles and by providing the structure within which valid inferential statements on a target population can be made and evaluated. Because we use parts of the whole, or samples from a population, we must make every effort to ensure, above all, that the resultant statistical samples will lend themselves to valid generalizations. The tendency discussed above for samples not to be representative of the population of reptiles under study is a problem because of the introduction of biases. Here, bias is used in two senses: first in the colloquial sense and secondly as a statistical term. In either or both cases, avoidance of bias is fundamental.

In the common, nontechnical sense, *bias* is a propensity for or inclination toward a determining influence on a decision. Bias can be positive or negative on a particular issue. In the technical sense, the definition has an additional, related component: bias results from the incorporation of systematic, not random, error. That is, "error" is the determining influence on the correct procedure. Random errors balance each other or average out; systematic or nonrandom errors cause a bias. Technically, bias is no longer associated with good or bad but only with an algebraic positive or negative influence on formulae. In the field, sampling is biased when subjective judgment overcomes the standard study design and the sample drawn is no longer free from nonrandom influences. The selection process must be unbiased, as must all procedures. Procedural biases can include incorrect definitions, investigator (or human) biases, and study design problems, among others. For example, let us imagine that an investigator selects a study area and lays out plots or sets traps with the aid of GIS (see **"**Using Geographical Information Systems to Design Reptile Surveys," above, in this chapter**)**. Especially with a relatively unknown area, it may be that the reptiles of interest are clearly more abundant just outside the boundaries of the selected study area. Unwittingly, the investigator has introduced a bias and, in general, will underestimate population size. Once the investigator recognizes this, she or he may wish to switch to ACS (see ACS under "Random Sampling: Unrestricted and Restricted," above), which can overcome many problems, including nonhomogeneous distribution of organisms. The best approach, of course, is for the investigator to carry out a preliminary field survey of the area, before selecting a specific study area. Bias can also enter into the statistical estimation or test procedures. For example, it is well known that an arithmetic mean is an unbiased estimator of the population mean; the variance formula is biased, but we can easily correct for that bias arithmetically. Some estimators are only unbiased in large samples and are called *asymptotically unbiased*.

Measurement error is the error resulting from methodological biases and errors, such as bias due to faulty gear, improper or inconsistent use of gear, improper training, human field errors, or natural variability that is not of interest to the study. Hayek and Heyer (2005) proposed a way to incorporate measurement error as well as effect size into the decision-making process for hypothesis testing when using amphibian morphometric variables; this method could also be used with reptiles. Measurement error is minimized with methodological standardization. As suggested in this manual, selection of cost-effective, low-variability sampling methods, proper training of personnel, standardization of methodology, and quality-assurance procedures designed to minimize methodological errors are all vital to the success of scientific field studies of biological diversity. In addition, measurement error can be reduced if multiple observations are made at each sample unit (e.g., in time, over depth or gradient, or spatially or temporally dispersed).

PSEUDOREPLICATION

Pseudoreplication is a term that has been popularized with respect to field data and/or the application of statistical methods that can lead to unwarranted inference. Hurlbert (1984) defined *pseudoreplication* as the use of inferential statistics to test for treatment effects with data from experiments where either treatments are not replicated (although samples may be) or experimental units are not statistically independent. It increases the risk of type-I error in any situation in which field data or statistical testing is used. Although this is touted as a common problem in ecological studies, pseudoreplication is neither "an error" nor "a bias." It is a general and, I believe, unfortunate term, because there is no such entity as a "pseudoreplicate". Strictly speaking, one does not "falsely" replicate a study or a factor. We either replicate, or we do not.

Hurlbert (1984) stated that the most common cause of pseudoreplication is carrying out repeated observations of the same individual and then treating those measurements as if they were of different individuals and thus were truly representative of the population. This action inflates the sample size and, in turn, the number of degrees of freedom upon which a test is based, thereby giving a statistical test more power than it should have. For a field study, pseudoreplication is easily avoided if the investigator does the following:

1. Recalls the difference between biological and statistical samples.
2. Develops specific questions in operational terms and then frames the relevant hypothesis to be testable and dependent upon the study's purpose (Hayek 1994a).
3. Obtains advice on statistical design before initiating the study.

An investigator must always specify the sampling unit when outlining the purpose of a field study. For example, for a density study the sampling unit is the quadrat or biological sample. For relative abundance (species proportions), however, the individual is the preferred sampling unit. Because cluster sampling uses a device (e.g., a quadrat), this device is the primary sampling unit for the field study, even if the investigator's interest lies in the individual. If an investigator specifies a question and determines the primary object of a monitoring effort, then the sampling unit becomes clear. Consequently, multiple observations on a single unit will not be considered independent, and the study will not involve what Hurlburt termed pseudoreplication. One either replicates, or one does not; one either repeatedly observes a sampling unit, or one does not. After the data are obtained, appropriate statistical treatments can be chosen, and inferences can be made.

An *independent sample* is one in which each data point in the sample represents an independent measurement. For example, we would not want to repeatedly sample the same individual or a single quadrat along a transect and consider those measurements to be independent. That procedure would not yield a sample that represents the target population, and as discussed above, when the sample unit is the quadrat, the individuals are counted but the number of quadrats constitutes the sample size. Note, however, that the problem of pseudoreplication is not the same as the often correct and desirable process of repeatedly measuring a sample individual over time.

RELIABILITY

Reliability is a measure of the constancy or stability of a trait or character. When monitoring over time or space, the characteristic being observed must be stable over that time or space. In addition, repeated measurements of the same sampling unit within a single study should yield consistent results if standardized techniques (as advocated in this text) are used and extraneous error minimized. Such traits are good characters to use in taxonomic studies or studies of variation. Snout-vent length of adult lizards and carapace length of reproductively mature turtles, for example, are good morphometric characters. Body weight in snakes, in contrast, is not a reliable character because it is situation dependent, varying according to how recently an animal has eaten and the size or number of its prey. Likewise, body weights may vary considerably according to current reproductive state.

Reliability is a property of a trait itself. In contrast, measurement error , which I discussed above (see "Bias, Measurement Error, and Biased Statistical Results"), denotes a lack of stability in the act of measuring or observing a trait that is stable. Investigators must always minimize measurement or observation error.

POWER AND EFFECT SIZE

Although research published in the reptile literature does not force the issue of power consideration, many other disciplines are adamant about its use. Indeed, funding agencies these days often require proposals to include a substantial power analysis. The concept of statistical power is widely familiar but often not well understood and thus ignored. Even general statistical texts, and certainly applied statistical texts, merely state the definition without providing additional explanation or relevant examples. *Statistical power,* in terms of probability, is a quantified measure of the ability of a statistical design to avoid making a *type-II error,* or *β error,* that is, of accepting a null hypothesis when it is false (in contrast, a *type-I error,* or *α error,* is rejecting a null hypothesis when it is true). More simply, Cohen (1977) defined statistical power as the probability that a statistical test will yield significant results. Unfortunately, many papers in the reptile literature contain statistical analyses with little ability to reject the null hypothesis when it is wrong (i.e., tests with low power). This probability, or power, depends upon both sample size and the ability to detect a true difference (i.e., an effect). In statistical terms, power depends upon at least four quantities: (1) sample size, (2) effect size, (3) type-I error level, and (4) sample variability. Although the values of these four elements may be known, the concept of power is not well integrated into the literature on reptiles.

Many times a reader or reviewer is surprised to see that two studies addressing the same question yield diametrically opposed results or reach opposite conclusions. One study accepts the hypothesis while the second study rejects it. Lack of power or excessive power is often the culprit. Without sufficient power, a researcher may not be able to recognize the effect of interest, that is, the biological effect that the study was designed to measure in the first place. With excessive power (i.e., too large a sample), just about any difference or effect, regardless of whether it is biologically substantive, will be called statistically significant. Of course, one may never see such conflicting results, because the "significant" result will be publishable, whereas the other often will not. Improper consideration of power can wreak havoc with any investigation. It may be that substantive differences exist but that the tests used to investigate the differences are not sensitive enough to detect them. Alternatively, there may be no substantive difference between two populations of geckos, one with a density of 21.04 individuals/ha versus a density of 20.96/ha. However, because the study design ignored power considerations and an extremely large sample was obtained and analyzed, the observed difference is called "significant." Investigators studying reptiles should pay more attention to power considerations and substantive effects and not concentrate merely on sample size or the *p* value.

Power can be analyzed either before or after data are collected. The analysis will help the investigator to assess the likelihood that the chosen study design will lead to rejection of the hypothesis being tested. If preliminary calculations show low power, then the study design should be revised or the significance of the test reconsidered. When revising a study design to increase power, the researcher must reconsider each of the four elements that affect power. Low power can occur if (1) the probability of making a type-I error is too high, (2) the variability of the data is too high, (3) the sample size is too small for the effect under test to be detected, or (4) the assumptions of the test are not adequately met. If, after the data have been collected, the power of a test is determined to be too low, the investigator must in the publication either declare the weakness of any inferences drawn or suggest that the study be repeated with a larger sample size. The costly result (an expensive type-II error) of a low-power study is, at best, its failure to increase understanding of the ecological processes involved (e.g., Reed and Blaustein 1995) and, at worst, support of decisions based upon incorrect study results (e.g., Peterman 1990). Many recent articles advocate the inclusion of statistical power analysis into any study design. Both statisticians and biologists appear to agree on the importance of *prospective power analysis,* that is, power analysis done before samples are taken. With that procedure, one can determine the sample size required to obtain unambiguous results. However, in fieldwork, a set sample size is neither always nor easily obtained. The alternative to prospective power analysis is *retrospective power analysis,* which is used to determine whether a body of fieldwork or an experiment is, in fact, powerful enough to have detected an effect. This type of power analysis is not usually recommended for many reasons, but primarily because the results will provide basically the same information as the hypothesis test's *p* value. So the questions of importance in fieldwork are how and when one actually controls power, and what alternatives are possible, which I discuss below.

An alternative to retrospective power, as well as an ingredient in power calculations is *effect size.* The size of an effect is a quantitative expression of the degree to which an alternative hypothesis departs from a null hypothesis. Effect size as a measure is a unit-free or dimensionless quantity, similar in this regard to a correlation coefficient. Different measures of effect size are used for retrospective and prospective consideration of the importance of an effect. For example, *Cohen's d* and *Cohen's f* (Cohen 1977), which can be calculated by most statistics programs (e.g., SPSS), are standardized mean differences. These values are used prospectively to calculate power and minimum sample size. Alternatively, the most popular measures for describing how meaningful a study result is in terms of the size of the biological or ecological effect are based upon the degree of association between the observed effect from the study and the dependent variable. When this type of association measure is squared, it gives the proportion of variance in one variable explained by the other, since each of the commonly used association measures are generalizations of the correlation coefficients r and r^2 (or R and R^2) from regression analysis. For example, for a test of population means, the prospective effect size (calculated before preliminary or final field sampling) would be the average difference between the two observed sample means from a pilot study divided by the standard deviation of this difference. Retrospective effect size is most commonly calculated by means of *Partial Eta Squared* (PES; available in statistics programs, e.g., SPSS). PES gives the contribution of each factor or interaction in an analysis, calculated as if the total variability were attributable to that factor, that is, as if it were the only factor. In fieldwork it is not always possible to design a study and predetermine the number of individuals or observations. However, I do recommend retrospective calculations of effect size. These values can provide a basis for further study by serving as preliminary values in a new study or for prospective power calculations (Hayek and Heyer 2005). That is, effect size is not equivalent to the probability level of a hypothesis test, in that obtaining a small p value (say, $p < 0.01$) does not imply that the study has detected a large or meaningful biological effect. Observing large changes in abundance (large effect size) may still only result in very minute changes in observed probability levels from the statistical test. However, the calculation of a measure of effect size will provide insight into whether the effect is actually large enough to be biologically or ecologically meaningful.

As I have discussed, a "significant" result statistically is not necessarily an important result biologically. Therefore, publication of scientific results should not merely concentrate on significance level. If each author cited either prospective or retrospective effect size and sample size, the reader would be able to assess the difference criterion and attribute substantive biological significance according to his or her expertise. In this way, the improper use of a statistical significance test would be easier to detect, and published results would become clearer and more comparable within specialty arenas.

Misinterpretation of Results

Many authors of papers that contain quantitative methods change terminology mid-stream and use colloquial terms in place of statistical terms. How often do we read in a paper that because $p < 0.05$ indicates a significant result, "the difference between scale row counts of two species, with $p = 0.10$," is not significant and then later in the same paper read, for example, that "it is 'significant' that the mean number of scale rows in one species of snake is higher than that of the second species in the same habitat." The interchange of scientific and colloquial use of technical terms is confusing and leads to interpretative errors.

Not surprisingly, problems with the interpretation of study results also arise when data are analyzed with the wrong statistical procedures. Misapplication of statistical tests and estimation techniques usually derives from a lack of understanding of assumptions or a lack of understanding of the statistical guidance obtained from a text or a person. Many mathematical statisticians are unable or, more likely, unwilling to delve deeply into a field of application that they only occasionally encounter. Alternatively, many "experts" whose statistical advice is sought are only peripherally involved in statistics; despite assuming the role of "statistician," these individuals try to practice statistics as a science but not, in addition, as an art. Decisions concerning substantive matters relative to the area of specialty of the investigator must be made before the objectivity of a statistical approach can be fruitfully applied.

In this section I discuss significance testing and correlation analysis, two statistical techniques that are most commonly and flagrantly misapplied, leading to rampant misinterpretation of study results. Reptile biologists are not the only offenders. Whenever studies of "natural populations" involve fieldwork, these two areas are problematic.

STATISTICAL VERSUS SUBSTANTIVE SIGNIFICANCE

I mentioned above the confusion of a colloquial term with a technical term. In particular, when "significance" or "significant" is used, a careful distinction is vital. In the statistical sense, these terms are a function of, always relate to, or depend on the size of the sample and the study design; they do not signify or determine the strength of some underlying relationship. The important question for fieldwork is not "Is the test result statistically significant?" If one takes a large enough sample, it assuredly will be. Rather, a researcher should ask "What size difference is important or meaningful for this study?" Couple this latter question with "Can my study design detect this size difference?" and the use of statistics becomes even more important to the reptile researcher. As Cohen (1977) emphasized, the most reasonable way to look at data is to express their summary results in terms of effect size. The importance of an effect size to the organisms being studied can be evaluated in a straightforward way; a significant test result cannot.

It is quite common to find that reptile (and other) researchers equate the importance of results with the number of "asterisks" in the table: that is, from most important *** ($p < 0.001$) to least important * ($p < 0.05$). This practice is incorrect. In fact, we can obtain more stars for any significant result merely by taking more observations. Unlike the determination of statistical significance, effect size incorporates both substantive and statistical elements, and its definition does not change as sample size increases. Use of effect size instead of reliance on the statistical significance of a test result will reassure a reader and advance scientific inquiry more directly. Can we deny the possibility that confusion exists concerning the application of statistical methods because only significant results are published? Following through on this proposition means simply that sample sizes for a particular study may be large enough to allow a difference to be detected, even if that difference has no biological meaning. Presentation of effect sizes determined either before or after the completion of the fieldwork would help to prevent meaningless results such as this, as well as statistical "lies." Whether it is appealing or not to believe, the monitoring of reptiles is a subjective chore. Statistics does not and cannot do all the work. Statistical significance does not evaluate the empirical importance of the test results or their biological meaningfulness. Including effect size and power information in a publication helps to alleviate confusion over whether or not results will be replicable.

THE *p* VALUE IN REPTILE RESEARCH

The traditional method for testing a hypothesis concerning reptile density, abundance, distribution, or diversity is to develop a rejection rule for which the probability of rejecting a true null hypothesis (type-I error) must not exceed some preset value, say, $\alpha = 0.05$. Unless the researcher parrots the commonly accepted values, setting the error level requires subjective judgment. Before the actual test, an investigator should predetermine, based upon expert knowledge of reptilian behavior, an acceptable probability level for making an inferential error, which is related to sampling error. The sampling error involved in each application can differ. Therefore, the error level can differ. In this way classical statistical theory is invoked, which minimizes the probability of a type-II error subject to the conditions imposed by the type-I (α) test-level selection.

Reliance upon computerized statistical packages, rather than tabled values, has led away from this procedure. It is common in current literature for the observed test value from the printout, say, $P = 0.0243$ (capital P is used for observed probability level, whereas lowercase p denotes the test level), to indicate the observed probability of the observed event. Only indirectly is the actual test level of, say, 0.05 considered. Many believe that this is perfectly acceptable because, of course, the result comes from the computer. However, some aspects of the practice are problematical. First, the interrelationships between both type-I and type-II errors and the power of the test have been ignored, and no minimization of errors, which is a basis for classical statistical hypothesis testing, is involved. The second difficulty is that the use of only the observed probability level permits each researcher to choose a unique level of significance quite arbitrarily, based upon his or her own single data set. Replication, therefore, may be problematical.

These issues can be resolved. First, for field studies, the choice of significance or test level usually is at best subjective and at worst arbitrary, subject only to mild convention and the dictates of an editor or reviewer. For scientific field research on reptiles, no "cost" for making statistical errors has been defined, nor are there exact specified consequences. However, we do have two issues of importance for the problem when we consider the formation of the null hypothesis. If the hypothesis concerns a discrete (not continuous) statistical distribution (say, a binomial or Poisson distribution), a selected test level may not even be possible (e.g., see Fisher's exact test in Hayek 1994b). Secondly, there may not be any information on alternative hypotheses about the field data, so a type-II error or a prospective effect-size value can only be a guess.

However, we can define this exemplar value of $P = 0.0243$ as the probability (under the null hypothesis) of obtaining a sample outcome equal to or more extreme than our observed sample. Such a statement can be more compelling than just providing significance at a stated rejection level ($p < 0.05$). In this way, our observed field samples are used to provide a measure of the probability level actually obtained out of the set of possibilities. Thus, if we consider as a maximum, a 5 percent rejection region in two tails, and our sample attains a 2 percent level (= 0.0243), we have more information to report than only the yes/no decisions on statistical significance. Coupled with information on effect size and the power of the test, the observed probability level can be especially valuable for field biodiversity data decision making. By using these statistics rather than presenting only the result of a significance test, we provide more reliable information on the reproducibility of the research for the reader. We can then ask if a roughly equivalent effect could be detected in additional studies under similar or differing conditions. Classical statistical tests do not provide this information.

CORRELATION, INDEPENDENCE, AND CAUSATION

Correlation is a word used regularly in the colloquial sense to mean relationship. For example, let us say we find a straight-line relationship between age and skin elasticity in *Boa* species. After measuring the variables, we can calculate a *correlation coefficient,* which is a quantitative evaluation of the strength of that relationship for that genus. Knowing that this relationship exists, we could estimate age given an index of elasticity or possibly predict elasticity knowing the age of a snake, but we cannot interchange either property relative to the other. We would never imagine that if we could increase the skin elasticity of a reptile, we could make it younger. To summarize, correlation is a measure of association, but association does not mean causation. When a statistical correlation coefficient is used as a quantitative measure of the joint relationship between two variables, the computation requires the input of observed values (measurements recorded) for two variables. The paired values of those variables are evaluated for their degree of relationship by computing a correlation coefficient. For continuous variables with a linear relationship, which encompasses most morphological, environmental, and density measurements, the *Pearson coefficient* is used most commonly to summarize the strength of the relationship.

Although a correlation coefficient can be calculated on any two sets of data this coefficient is, strictly speaking, applicable only to continuous, normally distributed data, such as density or transformed relative abundance. Only when linear data are normally distributed can we use the results for inferential purposes. The notation for Pearson's coefficient for sample data is either a lower case letter r or an r with subscripts indicating the names of the variables being correlated. For example, for variables denoted by x and y we use r_{xy}, whereas the Greek letter Δ or Δ_{xy} denotes the population parameter. The denominator of this coefficient is always positive; the numerator can be either positive or negative and determines whether the correlation value is positive or negative. Values of the coefficient range between +1 and –1, with 0 indicating a lack of association. Let us examine the implications of these limits. Let us say we want to use the density of one species to predict the density of another species that co-habits a particular forest environment. When the correlation coefficient is 0, which indicates a lack of linear association, we cannot predict the density of one species based on the density of the other because the first species provides no information about the second. However, "no information" applies only in a linear sense. That is, a zero correlation does not necessarily mean that there is no relationship; rather, it means that there is no linear relationship. A very strong nonlinear relationship could still produce a correlation coefficient of 0. Alternatively, if the value of a coefficient is –1 or +1, then the observations on one species perfectly describe the observations for other. Thus, there is actually no need to know both sets of data since all information is contained in the single species data.

If a correlation is perfect (i.e., if the coefficient is positive or negative 1), the values for the two variables should lie in a straight line when plotted. Construction of a scatter plot allows the investigator to see that. If the variables are directly related, then the plot will climb toward the upper right of the graph, and the value of the calculated correlation coefficient will be positive. If instead the variables are inversely related and the plot values go downward toward the lower right area of the graph, with low values of one variable associated with high values of the other, then the coefficient will be negative. It is

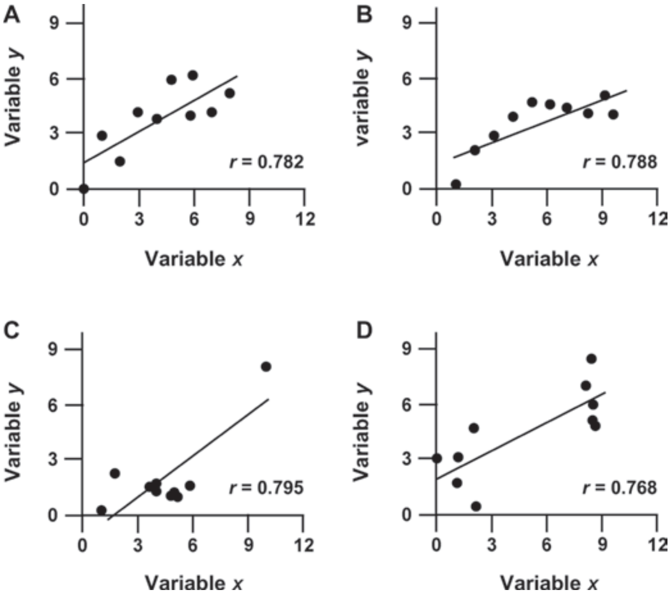


FIGURE 7 Graphs of two variables whose relationships are described by the calculated Pearson correlation coefficient of r=approximately 0.8. In (A), the variables have a linear relationship, and r is 0.8, indicating how tightly the data congregate about the line. In (B), although the variables have a strong curvilinear relationship, r=0.8. In (C), the data group into one homogenous subset with r=0.2 and a single outlier. When the outlier is included with all other points, r=0.8. In (D), the data fall into two distinct groups; the correlation of all points, without recognition of group membership, is r=0.8. In the latter three examples (B–D), r has no meaning in relation to the straight line. Often, relationships can be confused when only a single statistic is used to describe them, and without a picture, an investigator may not be able to detect the true relationship between variables.

important to realize that this association holds "on average." It will still be fairly simple to find pairs of individuals or observations whose relationship is not defined by the coefficient. Consider four scatter graphs (Fig. 7), each of which represents approximately the same strength of association between the variables, with an r of about 0.8.

These plots may shock you. Just as with any single index or summary statistic, for example, those for diversity (Hayek and Buzas 2010) or similarity (Hayek 1994b), a variety of data situations and relationships can be combined or confused when only a single statistic is used to describe them. For this reason, I strongly recommend that a scatter plot always be provided in addition to a simple r value. The failure of the correlation coefficient to distinguish the characteristics of each of the plots highlights certain well-known errors that result from the misapplication or interpretation of correlation analyses. The errors relate to (1) attenuation, (2) nonlinearly related variables, (3) outliers, (4) group versus individual relationships, and (5) spurious correlations. I will review some facts about correlation coefficients and then discuss common errors involved with their application to data.

It is important to understand that it may not be possible to attain the theoretical limits of +1 and –1 for a particular sample data set. This is called the problem of *attenuation*. If the range or variance of one variable or both is truncated or very narrow (say, garter snake densities from habitat 1, which range between 9.1 and 10.5 individuals per quadrat , are to be correlated with those from habitat 2, which range from 2.8 to 40.9), the value of the correlation coefficient will never be able to attain its theoretical limits. This may have grave implications. A calculated value for r of 0.2, for example, appears to indicate minimal correlation. But, if the attenuation is severe and for your particular data set the maximal possible value of r is only 0.56, then the correct interpretation of the result clearly is difficult. A correction for attenuation can be found in any elementary statistical text or applied by most computer statistics packages for regression or the general linear model. Although a correlation coefficient is a decimal value, it is not a proportion, and multiplying the value of r by 100 does not produce a percentage of some whole. The percentage of variation in one variable that is explained by the other variable is equivalent to r^2 multiplied by 100. This is called the *coefficient of determination* and denoted by R^2.

It is not unusual for biologists to assume that the absolute value of the correlation coefficient is an indicator of the strength of the relationship between the variables. However, because r is a multiplicative average of both variables, we cannot say which cases or what proportion of cases contributed to the relationship and determined the magnitude of the value. Consider Figure 7A. If a particular coefficient is large, then the data points will be tightly concentrated about a straight line drawn through the data on the plot. For a value larger than 0.8, the points should be even more tightly grouped about the straight line. Although this is generally true, it does not apply in every case. First and most importantly, the data have to be linear. Without linearity, the calculated value of the coefficient will have no meaning in relation to a straight line and will not indicate how tightly the data congregate about the line. This is illustrated in Figure 7B, where $r = 0.8$, just as it does in Figure 7A. Without a picture, an investigator would remain ignorant of the true relationship between the variables, assuming that the (correctly calculated) value of r indicates the existence of a meaningful linear relationship.

Researchers should be aware that adding a constant to each observed value or multiplying it by a constant will not change the value of the correlation coefficient. In fact, the value of the correlation coefficient does not change as the result of any linear transformation unless one set of values is multiplied by a negative number, which will change the sign but not the value. For example, measure a variable in inches or centimeters, and the value of the coefficient remains the same. Make an error and record each observation in millimeters instead of centimeters, and the final value of the coefficient will not be affected. Correlate the weight in kilograms or in pounds with length, and the resultant coefficients will be identical. However, the correlation will change if the data are log transformed, because a logarithm is not a linear measure.

Let us now examine the case of *outliers,* or data points that lie far outside the range spanned by the other data points. Consider the scatter diagram in Figure 7C. The correlation coefficient calculated on just the set of nine points (pairs of x-y values) in the lower left of the graph is only 0.2. If the correlation is recalculated based on all the points, the value of r is 0.8. This is because the point in the upper right falls so far from the rest of the points, that it is considered to be an outlier. This outlier increases the means of x and y as well as the value of the correlation coefficient. Reporting the higher coefficient without mentioning the outlier would be misleading. It is always easier to detect such a distant point when the data are plotted. Outliers are common in data that have decimal points; often the decimal point in one of a pair of observations may have been recorded incorrectly. Irregular failures of measuring or recording equipment and/or investigator carelessness can also produce outliers. Alternatively, an outlier can be a legitimate observation and must not be arbitrarily omitted, as mentioned previously (see "Pseudorandom Sampling and Truncation," above).

If samples are small, r is less accurate. However, an aberrant point in a small sample has a higher probability of being a legitimate population data point than does a deviant point in a larger sample, because fewer representatives are available to characterize the natural population. It is even possible that the one aberrant point reflects the population mean and that the cluster of points is an atypical grouping. Outliers always make r higher or lower. It is vital to a study, therefore, to examine each such observation and to argue for its inclusion or elimination in the published report.

Figure 7D illustrates a problem associated with grouped data. The correlation between two groups is not necessarily mirrored in the correlation among all the individuals from each group. Without a scatter plot, the investigator would believe that the observations were highly correlated because $r = 0.8$; yet Figure 7D shows that the actual relationship is between the two points that are the means of each group, not among the individuals within each group. If the correlation is in the same direction (in this case positive) for both individuals and groups, the resultant coefficient, based on averages, typically will overestimate the strength of the individuals' relationship because, in effect, the computation eliminates the variability within the groups.

Even with a truly random sample, interpreting the results of a correlation analysis may be difficult. It is always possible that the relationship between two variables with a high correlation coefficient is spurious. The relationship observed may actually be caused by one or more unobserved factors. For example, the large negative r value for the abundances of two species of lizards along an elevation gradient may imply correlation but may in fact result from the correlation of abundance of one species with cooler temperatures and the totally independent correlation of the abundance of the other species with warmer temperatures. Environmental variables are usually recorded and examined along with field counts of organisms. It is often thought that such variables directly influence field data through their effects on habitat productivity, community cohesion, or simply animal activity patterns. Apparent associations may be recognized and true associations missed because data on the appropriate variables have not been recorded. Spurious correlations are common when data sets include outliers or unrecognized data groupings, which is another strong reason to plot the data before analysis.

If data are collected over any length of time, then the data points or observations may be temporally correlated. This type of correlation increases the variance of the distribution and will result in larger than expected correlations. The statistical procedure used for detecting an extraneous variable(s) is multiple regression, which incorporates partial correlation and can describe the amount of the correlation attributable to each variable or the degree to which variables are correlated with each other. Observed relationships that are suspect (e.g., a large increase in density over time) can be investigated with the partial correlation procedure. Any researcher suspecting such a problem should seek the advice of a professional statistician, because partial correlation analysis can easily lead the unsuspecting to illogical conclusions and unreliable, irreproducible results.

A correlation coefficient can be calculated for any two sets of data; no assumptions are necessary for the calculation, as mentioned above. However, the tabled value will denote the correct significance level of a calculated value only if the variables are independent and normally distributed. Only under those conditions can an investigator draw legitimate inferences from the data. Equally important is the fact that even though a value of r can be reported, its proper interpretation depends on the sampling scheme used to obtain the data. Investigators should keep in mind that it is best if both sets of observations are based on random (unrestricted or restricted) samples from the same population, because then r estimates the population coefficient. Only after that can two or more correlation coefficients be compared. Also, the accuracy of a calculated r value depends upon the sample size; larger samples yield more accurate measures of correlations. Even with large samples, measurement error in either variable will affect the final result. Because an error may be in either direction, the resultant r can be an overestimate or an underestimate, with no indication as to which. Unfortunately, many researchers fear that their samples are too variable to be proper subjects for statistical analysis. They fail to recognize that the opposite is also problematical; if a sample is too homogenous and spans too small a range, the estimate will be biased, and r will be underestimated. Finally, without a scatter plot of the observations, the investigator will not be able to detect any problematical data points, errors in assumptions, or groupings in the data that can affect the interpretation of a coefficient and the conclusions drawn from the study.

CHAPTER FOUR

Dealing with Associated Data

MERCEDES S. FOSTER and ROBERT N. FISHER
Chapter Coordinators

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Overview

Mercedes S. Foster

When designing an inventory or monitoring project, investigators rightly tend to concentrate on the "Big Issues" much the way a journalist might, determining the who, what, where, and why of the project. To do so most effectively, the investigator must be very clear about the goals of a study and what he or she is trying to learn. With that in mind it is possible to select (1) the most appropriate organisms to study, (2) the optimal site(s) for carrying out the work, (3) the sampling methods that will provide the type and amount of data required to answer the question(s) being asked and will be appropriate for statistical analyses, and (4) how the data will be analyzed and disseminated. Although such a focus is to be expected, it is important for investigators to spread their nets a bit wider to consider ancillary factors that may have indirect but profound effects on the outcome of an inventory or monitoring program and that may explain the data that are obtained.

In the first part of this chapter, the authors consider various types of ancillary data. Information about climate and weather is basic. Temperature and rainfall immediately come to mind, but other weather variables, such as wind speed and direction or humidity, may also be required. Equally important are data that describe the habitat, either terrestrial or aquatic. For terrestrial environments, vegetation structure is of primary concern. Unfortunately, the author who agreed to prepare a section on habitat sampling for this volume did not complete his contribution. Fortunately, multiple protocols for describing habitats and vegetation are available, both in published form (e.g., James and Shugart 1970; Cooperrider et al. 1986) and on the Web. Habitat sampling can run from the simple to the very complex, employing satellite images and aerial photos, topographical and geologic maps, and various classification schemes. However, as an adjunct to a survey of reptile biodiversity, basic but quantitative approaches are often adequate. Physical aspects of the environment (e.g., soil type, slope, exposure, pH, dissolved nutrients, and so forth) can be sampled as well if they are relevant to the study and/or study organisms. Data on soil characteristics may be essential to studies of fossorial and burrowing snakes, but not a top priority for studies of arboreal lizards. Once the general habitat has been described, microhabitat data are useful for explaining the distributions and abundances of animals within it.

Once an investigator has determined which data are to be collected, he or she must determine how best to record and store them and ensure their quality, issues considered in the latter half of the chapter. Nowadays, it almost seems as if investigators can measure and record an environmental variable and record the data with anything from a cell phone to a sophisticated electronic device and then link to a computer to download the information automatically. Even in the absence of the investigator, some devices can send automatically recorded data via satellite link directly to a computer in the investigator's laboratory. Nevertheless, "smart" electronic instruments can be expensive, heavy, sensitive to environmental conditions (especially high humidity), and require an external power source, so investigators must think carefully about which instruments are appropriate in the context of their budgets and the location and conditions at the study site. Running

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to the corner electronics store for replacement parts may not be an option. If possible, investigators should always carry alternative measuring and recording devices—pencil, paper, and a ruler are a must!

Climate Data and Seasonality

Joseph C. Mitchell

Ectothermic reptiles respond directly to changes in climate and weather. Their responses to these environmental factors influence whether or not one encounters the animals during biodiversity surveys. Consequently, survey designs must take into account the characteristic climate of the target region and the seasonal activity patterns of reptiles that occur there. Designing a study to coincide with the time of year during which the target species are most active or most likely to be encountered can minimize the effects of climate and season, and help to ensure unbiased results.

How climatic and seasonal factors affect results of biodiversity research depends on the location of the project and the species under study. Proximate factors to which all reptiles respond are temperature and rainfall. These are, in turn, affected by latitude, elevation, ecosystem, habitat, global climate cycles, and global perturbations (e.g., El Niño, global warming). Most readers are aware that activity patterns of reptiles in the tropics differ from those of reptiles in temperate zones. Even temperate zone activity patterns vary within species both seasonally and annually and may affect one's ability to encounter these animals on a regular basis.

The seasonal activity patterns of reptiles vary considerably both within and among regions. Sympatric lizards in North America differ widely in the number of months in which they are active (Etheridge and Wit 1993). Activity patterns of desert lizards, for example, often differ between dry and wet months (e.g., Whitford and Creusere 1977). Most species of temperate-zone snakes exhibit one of two primary patterns of annual activity: unimodal, with a single peak between late spring and late summer, and bimodal, with one activity peak in the spring and another in the fall (Gibbons and Semlitsch 1987). Seasonal patterns of activity for tropical lizards and snakes are much more variable, even among species in the same location, as some show wet and dry season differences and others do not (Barbault 1976).

Many species of reptiles also shift activity patterns within seasons in response to temperature changes and thermoregulatory needs. For example, Australian tiger snakes and some temperate-zone watersnakes change from daytime activity to nocturnal activity as the temperatures increase in summer months (Mushinsky and Hebrard 1977; Schwaner 1989). Gravid pitvipers in eastern North America move to open habitats with higher temperatures, whereas nongravid females and males remain in less open, cooler environments (Reinert 1984). Desert lizards shift from being active in the middle of the day early in the year to primarily morning hours during hot summer months (Pianka 1986).

Periodic variation in climatic conditions can directly affect animal activity and population demographics. Drought, for example, has led to emigration from wetland habitats, population decline and extinction, and altered population structure in temperate-zone snakes (Blair 1957; Dodd 1993a; Seigel, Gibbons, and Lynch 1995). Severe flooding caused changes in sex ratio and population structure in an endangered population of rattlesnake (Seigel et al. 1998). Other factors influenced by climate and weather, such as prey availability and ecological succession, affect annual variation in the population sizes of individual species of lizards and snakes in temperate zones and the tropics (Andrews and Wright 1994; Fitch 1999).

Because climate and weather events play such important roles in the ecology of reptiles, successful inventory and monitoring projects should include an evaluation of the environmental conditions of the study area. Understanding regional climate conditions and how target reptiles respond to changes in weather allows researchers to implement those standardized methodologies that increase the probability of encountering the target species. In addition, the results obtained during actual operation of a monitoring project will depend, in part, on climate patterns and weather conditions during the sampling period. Basic weather data is essential because changes in activity patterns and abundances can be evaluated only if one understands differences in temperature, rainfall, and other physical factors. Thus, data on the following parameters allow one to evaluate the results of a monitoring project in an environmentally realistic context.

Temperature

The most important aspect of taking temperature and other types of physical data in one's study or survey is consistency. If possible, temperatures should always be taken with the same instrument in the same microhabitats. The general standard is to take air temperatures 1 m above the ground, in shade. Time of day and microhabitat should always be recorded, as well as a description of the current weather conditions. Basic air and water temperatures can be obtained with a simple centigrade mercury thermometer. The one that has been most often used for reptile research is the Schultheis (Miller & Weber; see Appendix II), quick-reading cloacal thermometer. Although designed for taking animal temperatures, it can also be used to take air and water temperatures. Several other instruments available greatly extend the range of environmental data that can be obtained. Researchers should review available instruments to determine which may be best for their programs within the context of time, budget, and research questions.

Mercury thermometers are commonly available, although water pollution from mercury contamination has become a concern in many areas. Several thermometers that are now available use other fluids and are just as accurate, but less polluting. A max/min thermometer is useful for recording daily maximum and minimum temperatures, assuming that it can be reset each day. If the thermometer is placed within a slotted wooden box, the temperatures obtained accurately reflect outside temperatures because they are not influenced by reflective surfaces. Electronic thermometers are relatively inexpensive and usually provide accurate readings. Digital temperature dataloggers are now common and available in a range of sizes and configurations to record a wide variety of environmental temperatures. The small ones are particularly useful for obtaining data from microhabitats that previously could not be evaluated. Dataloggers record temperatures at any time interval needed by the researcher to meet his or her research goals. They can be launched by computer from the laboratory or office. Files can be downloaded from a datalogger in the field with an electronic handheld shuttle, which eliminates the need to take a laptop into the field or bring the datalogger into the laboratory. The shuttle also resets the datalogger. The shuttle, in turn, connects to the laboratory computer, and the data are entered into spreadsheets for analysis. Data are presented in graphic form, but the numerical information is also stored in a file that can be manipulated by database software (e.g., Excel). The only difficulties I have experienced with such devices are insufficient battery life and destruction by timber-logging operations. If a datalogger has been sitting on the shelf for some time, it is wise to replace the batteries with fresh ones before deployment. Finally, if security is a concern at the field site, it may be prudent to hide the dataloggers from view.

Precipitation

Reptiles often respond quickly to rainfall events, and periodic activity cycles are determined by wet/dry conditions that may be correlated with temperature. Data on weekly, daily, or hourly rainfall amounts often prove essential for describing and evaluating observed reptile activity patterns. Precipitation data can be obtained in a variety of ways. The simplest is to deploy a standard rain gauge in the study area and record the data at the same time each day. If it is not always possible to be on site at the same time each day, then the data must be obtained by other means.

Rainfall dataloggers are a reliable way to obtain precipitation data without having to be on site. Digital data on amounts, rates, and duration of rain, as well as a date and time stamp, can be downloaded to a shuttle and uploaded into a laboratory computer for analysis. These instruments are available from most biological and field supply companies (see Appendix II). Several companies make rainfall dataloggers with a variety of different benefits and costs. This relatively new way of obtaining precipitation data has many advantages over the manual and remote methods that we used in the past, and it is worth exploring if funds are available. As with the temperature dataloggers, security may be an issue.

In the United States, the National Weather Service (see Appendix II) has been accumulating environmental data for more than a century. The data are recorded at permanent weather stations, some of which are checked daily by interested citizens. The data, including daily rainfall, are readily available via subscription to monthly bulletins or on the Internet. Unfortunately, these weather stations are not always located near one's study area and may or may not accurately reflect precipitation levels at the specific study site. Unless a permanent weather station is located at the study site, weather data obtained from such stations should be used with caution and only to describe local patterns; they are generally not appropriate for site-specific, cause-and-effect evaluations of reptile behavior.

Other Environmental Data

Numerous other types of physical data may be useful for the study of reptile activity patterns. These include humidity, wind speed, duration of sunshine, sunlight energy, water turbidity and flow rate, pH, salinity, soil temperature and moisture, slope angle, and canopy cover. Researchers should evaluate the types of instrumentation that are available, affordable, and accurate. Advice from colleagues who have had experience collecting data on various physical characteristics of the environment can be extremely helpful. Accuracy of inferences made from instrument-based measurements depends on what is meaningful to the species under study. Knowledge of instrument calibration is also extremely important and must be appropriate for the target animal. For example, standard light meters were originally calibrated in 1923, in lux, based on the spectral responses of 52 American humans (Jones and Reynolds 1996). However, the sensitivity of reptile skin to light wavelengths is not the same as that of human skin. Worldwide websites such as WORLDCLIM (Hijmans et al. 2005) offer a variety of environmental variables for many places in the world that may be useful for ecological niche models and asking questions about reptile activity patterns in nature.

In summary, when deciding on the kinds of environmental data to be recorded during a study, researchers should consider both the physical environment and the natural history of the target species and then ask what types of data would be most relevant to the target species. Investigators should plan ahead, know their instruments, and know their animals.

Describing Microhabitats

Kim Gray-Lovich and Robert E. Lovich

We use the term *macrohabitat* to refer to broad habitat types (e.g., primary tropical forest, desert scrub, riparian, etc.) comprising an area of interest. We use *microhabitat* to refer more specifically to the particular habitat being utilized by a species on a smaller scale. Microhabitats can be defined as areas within general, large-scale habitat types that are characterized by the specific set of condition required (or preferred) by a particular reptile species. They are subsets of large-scale habitat designations, at a much finer resolution. Our goal in this section is to help researchers understand the importance of microhabitat data, how they can enhance the quality of a study of the target reptile species by recording such data, and how standardized methods of collecting microhabitat data can make this task easier to accomplish.

Microhabitat data recorded during a project will increase the "power" of any study considerably. The Desert Night Lizard (*Xantusia vigilis*), for example, was first discovered by the American naturalist John Xantus and subsequently described by Spencer Baird (1859). For 30 years following its formal description, the lizard was found so infrequently that it was considered "rare." However, once investigators discovered that its preferred microhabitat was downed yucca (*Yucca brevifolia*) plants, a microhabitat previously overlooked for this species, they determined that this lizard represents the highest single species biomass in some desert areas it inhabits (Bezy 1988)!

Recording microhabitat data should be a requirement for virtually any type of reptilian study. While the general habitat requirements for most reptiles may be understood, microhabitat affinities for most species are poorly known. Microhabitat requirements may change at various stages through the life cycle of a reptile, differ between the sexes or phenotypic variants of a single species, or vary across its geographic range. Preferred habitats (and the species inhabiting them) are usually unevenly distributed and have certain features that set them apart from adjacent habitats. Why a species prefers a particular location within a larger habitat type may not always be apparent. As with the example of *X. vigilis,* later work on a target species may show that earlier data could have been extremely useful (e.g., for *X. vigilis,* a note that initial specimens were collected in downed yucca), although their importance was not fully understood at the time of the original data collection. For this reason, it is always important to gather as many data as possible on microhabitats and their characteristics.

Reptile microhabitats include a multitude of smaller-than-ecosystem-scale habitat components such as soil type, substrate type, specific vegetation type, slope, temperature, aspect, and rainfall. Microhabitat data for aquatic reptiles additionally can include variables related to the water, such as pH, dissolved oxygen (DO) content, salinity, depth, flow rate, form (e.g., river, pool, reef), and many other characteristics. The presence (or absence) of each component and their descriptions collectively make up the description of the microhabitat. In addition, how an animal uses a microhabitat or one of its components and its behavior in that microhabit are recorded.

Knowing the circumscribed distribution of the focal species being surveyed may allow a researcher to locate it more easily. However, reptiles are often unevenly distributed and prefer specific subhabitats within their given ranges. Some questions to consider in this respect are:

1. What diversity of physical characteristics exists within the circumscribed range of a species?
2. What physical or other characteristics distinguish the microhabitat from surrounding or other microhabitats?
3. How does the target species use the microhabitat?
4. Is there a pattern evident in the distribution of individuals of the target species in the habitat?
5. Do individuals in the target-population habitat share areas or other resources?
6. Does the use of microhabitats change by season or across years?

Understanding the answers to these questions can help an investigator develop a list of microhabitat attributes that are important to a focal species or group of species and to focus his or her time and efforts more effectively on those species.

Specific Needs and Requirements of Reptiles

Reptiles, as with all living organisms, require certain resources in order to survive. Food, water, and shelter are obvious essentials, but other requirements must also be met if the animals are to reproduce and thrive. Every given habitat has specific components that determine its "habitability" for the resident reptile fauna. Secure, undisturbed sites for hibernacula; a specific substrate type; egg laying sites with appropriate temperatures; adequate basking and cover sites; and productive foraging sites are some examples. Each species of reptile requires a microhabitat with a unique suite of characteristics. When determining or describing the microhabitat needs of a reptile, one must consider aspects of the natural history of the species. For example:

1. Does the species lay eggs or give live birth?
2. What are the main prey items of the species?
3. What is its primary living habit (e.g., fossorial, arboreal)?
4. Does it depend on moisture (e.g., mesophilic), and if so, how much is required?

Such questions will help the researcher establish what may be "good" habitat for the species of reptile being studied.

Indicator Species

Locating focal species when carrying out a research project is not always easy. Knowing which species occur in association with one another, however, can be just as informative as finding the target reptile (e.g., when surveying potentially "good" habitat). Some reptiles are species specific when it comes to animal prey or plant food items; others are often found in the presence of another species. For example, *Phrynosoma* spp. and native ants are frequently associated, as are Gopher Tortoise (*Gopherus polyphemus*) burrows and Eastern Diamond-backed Rattlesnakes (*Crotalus adamanteus*). Knowing which species may be indicators of another species, as well as the abundance of the indicators in a particular area, may help a researcher to assess the potential for the presence (or absence) of the other species in that area.

Sample Data for Microhabitats

GENERAL INFORMATION

- Date
- General location
- Time
- Latitude and longitude (with Global Positioning System)
- Weather
- General vegetation
- Aquatic or terrestrial
- Exact cover type (rock type, soil type, vegetation type, etc.)
- Canopy overhead (e.g., dense closed canopy; open partial canopy; completely open)
- Soil/substrate type (e.g., rocky soil, leafy debris, sand, moss, etc.)
- Potential impacts (e.g., exotic plants or animals, development, land conversion, etc., nearby or in close proximity)
- Habitat status (e.g., degraded/disturbed, natural/undisturbed)
- Other species of reptile nearby or in the same location
- Activity in which the reptile is engaged (if observed)
- Method of trapping (e.g., pitfall, snake/funnel trap, noose, sticky trap, etc.) if samples or specimens are collected

MICROHABITAT DESCRIPTORS AND ANIMAL LOCATIONS BY HABITAT TYPE

DESERT OR SCRUB HABITAT

- Rock outcrop
  - concealed under/beneath rock
  - between rocks
  - basking on rock
  - size of outcrop
  - type and size of rock
- Burrow
  - type of burrow (e.g., mammal, reptile)
  - size (length, diameter of opening)
  - substrate

  - angle of descent
  - animal location (e.g., completely within, at entrance)
- Scrub
  - type of vegetation (e.g., cactus, sagebrush, creosote scrub)
  - animal location (e.g., at base of vegetation, in vegetation and height, etc.)
- Dune or wash
  - substrate type
  - animal location (e.g., on slope, in open, partially or completely buried, etc.)

WOODLAND OR RIPARIAN HABITAT

- Rock outcrop
  - concealed under/beneath rock
  - between rocks
  - basking on rock
  - size of outcrop
  - type and size of rock
- Downed wood
  - under log/bark
  - in rotting log
  - on log/bark
- Shrub or tree
  - in tree/vegetation (height; diameter of perch)
  - in tree cavity
  - among roots
  - in leaf litter at base of tree/vegetation

GRASSLAND OR MEADOW HABITAT

- Grassy clump
  - type of grass
  - at base of grass
  - vertically in grass (height)
- Open meadow
  - facing up or down slope
  - near or away from edge
  - type of vegetation or substrate
- Rock outcrop
  - concealed under/beneath rock
  - between rocks
  - basking on rock
  - size of outcrop
  - type and size of rock

POND OR MARSH HABITAT

- Waters edge
  - on bank
  - near edge in water
- On vegetation
  - partially submerged log
  - reeds in water/on bank
  - type of vegetation (e.g., reed, sedge, willow)
- In water
  - depth below surface
  - in aquatic vegetation
  - partially or completely buried in bottom substrate

MICROHABITAT USE

Information regarding the activity of the reptile in a particular microhabitat may be quite valuable when interpreting the overall microhabitat requirements of a species. Examples of behavioral data that should be recorded are as follows:

- Resting (not basking)
- Basking
- Actively moving (not foraging)
- Burrowing
- Climbing
- Refuging/hiding
- Foraging
- Displaying territorial or threat behavior
- Displaying courtship behavior
- Nesting

Diversity, Distribution Maps, and Atlas Production

Ralph W. Axtell

A demonstration of the organismal diversity of any area requires substantial knowledge of the occurrence of each organism in nature. Word descriptions provide only a generalized picture of such distributions, so maps are used to fill the informational void. Most distributional maps used today apparently originated and evolved in two ways. The first crude distribution maps (mostly of birds and large mammals) seem to have been based on observations alone. A biogeographer would select a base map (usually of continental size, but sometimes smaller) and then simply estimate boundaries of a species' range based on then current knowledge of general areas where the organism had been seen. The second (and slightly later) method of map production was apparently based on actual observation or collection sites, which were plotted on a personal road map or other larger-scale map and then transferred as outlines or spots to a simplified base map by photographic or other means. The earliest use of spot maps in herpetology that I have found is Blanchard (1921) in his revision of the kingsnakes (*Lampropeltis* spp.). Maps of both of these original types were modified piecemeal as additional information became available.

Contemporary maps of animal distributions come in many sizes, styles, and shapes—some good, some bad; nevertheless, most convey much more information than do words. Interestingly, almost all contemporary maps portray only a single taxon, a practice that tends to obscure associations with other species, especially those that are closely related. Below I review several map styles that are still used. I point out their good and bad aspects and briefly discuss why they should or should not be used in diversity studies.

Map Styles

BALLOON DISTRIBUTION MAPS

This type of map, which is used in field guides, books, and in many scientific studies, is essentially an outline map with a minimum of reference points. Usually only continental and state boundaries are shown, although larger rivers are often added.

PROS These maps are fine for field guides, giving the amateur some idea of where an organism can be found.

CONS It is often implied that such maps represent reality, which they do not. Because only an external boundary is present (and this may be partly conjectural), they show no (or very few) perturbations along or within that boundary.

USE IN DIVERSITY STUDIES These maps are much too general to use in diversity studies, especially those conducted on a smaller scale. On a global or continental scale they may be appropriate, depending on map size.

COUNTY DISTRIBUTION MAPS

These maps are similar to the balloon map, but on a smaller scale (e.g., in the United States, usually state size). Shading or spotting (but usually little else) indicates that the organism occurs in individual counties (Fig. 8). This map type has become popular recently, especially in state field guides.

PROS Such maps are inexpensive and easy to produce, and they encourage younger (and some older) herpetologists to publish new county records in *Herpetological Review* or elsewhere.

CONS If such maps are used to infer range boundaries (often the case), distortion can be substantial. In some cases only a small portion of a county may be inhabited, yet the map and the boundary often include the entire county. Owen and Dixon (1989), for example, used this map style in an ecogeographic analysis of the Texas herpetofauna. Their working "diagrams" were so distorted and confusing that Ward et al. (1990) found it necessary to publish an alternative ecogeographic view based on essentially the same data.

USE IN DIVERSITY STUDIES These maps are much too crude for use in diversity studies. Visualize, for example, such a map for the states of Arizona or New Mexico, where the counties are extremely large. Range boundaries determined using such large patterns would be virtually meaningless.

PERIPHERAL DISTRIBUTION SPOT MAPS

These maps are similar to the balloon map in depicting only an outline boundary, although its position is based on published peripheral localities (= spots) where vouchers have been taken. I know of no herpetological maps of this genre, but all maps in E. R. Hall's *Mammals of North America* (Hall 1981) are of this type.

PROS These maps show marginal distributional boundaries slightly more accurately than do the two map types mentioned above.

CONS A major criticism of these maps is their neglect of areas inside the external boundaries, which may be important. Also, many of the locality points may be too far apart to portray much local or regional variation accurately.

USE IN DIVERSITY STUDIES Not recommended, for the same reasons that county distribution maps are not recommended.

TRADITIONAL SPOT MAPS

These maps show state and/or county boundaries and larger streams, as well as localities from which vouchers have been collected, using spots of various sizes to indicate numbers of specimens taken. Such maps are usually based on museum or collection data plotted by eye, scale measurements from reference localities, or *Global Positioning System* (GPS) data. External and/or internal boundaries can easily be added to these maps to produce sophisticated and more accurate balloon maps.

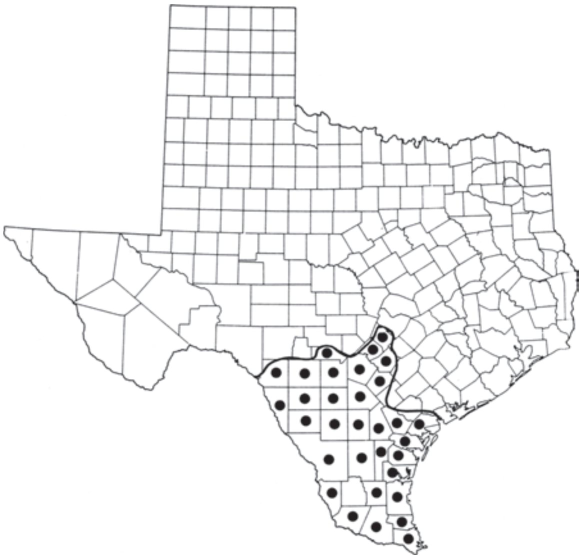

FIGURE 8 Example of a county distribution map. Note that the distributional margins are largely based on county boundaries. (Reprinted, with permission, from Dixon 2000, map 84 © Texas A&M University Press.)

PROS Internal and external structure can be interpreted if enough records are available. Each point on the map can also be reproduced for verification.

CONS With just state or county boundaries shown, interpretation of a distribution is severely limited. The addition of major physiographic and geologic features, streams, soil types, and vegetation greatly enhance the utility of this map type.

USE IN DIVERSITY STUDIES Such maps are minimally acceptable for diversity studies, but their use should be limited to fairly large areas.

GRID DISTRIBUTION MAPS

This map is similar to the county distribution maps, except that a measured grid with considerably smaller cells is laid over the targeted range. A dot is placed in each grid quadrangle within which the organism is known to occur (Fig. 9). All grid cells are of equal size, but they may vary from map to map.

PROS These maps are an improvement over county distribution maps because they show both a boundary and internal structure.

CONS Unless the grid cells are very small, these maps suffer the same inaccuracies as do county maps. Also, such maps are less esthetically pleasing than traditional spot maps because the spots are arranged in rows and columns instead of as random punctations in areas of occurrence.

USE IN DIVERSITY STUDIES European biogeographers use these maps most frequently. Because the spots on the map are centered within the grid quadrangles and not on actual collection sites, the maps are not as accurate as traditional spot maps.

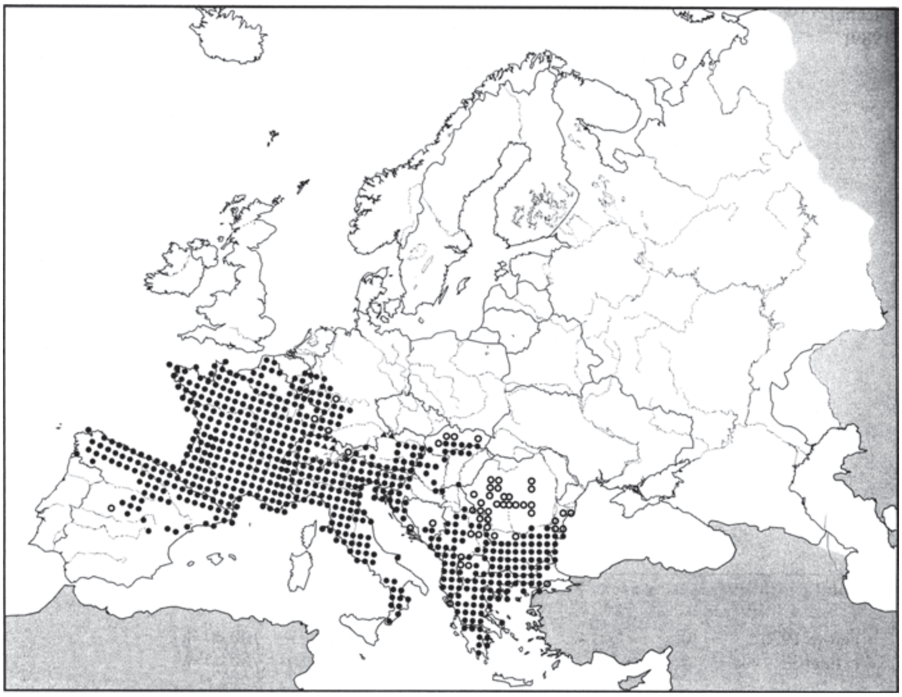

FIGURE 9 Example of a grid distribution map. Note that the dots and therefore the grid squares are too large to achieve much more than a basic distribution pattern. The grids do not allow much internal or external structure to the distribution. Printing register problems have left some of the distribution off the coast in saltwater. (Reprinted, with permission, from Gasc, J.-P. et al. [eds.]. 1997. *Atlas of Amphibians and Reptiles in Europe,* p. 286 *Podarcis muralis*. Societas Europaea Herpetologica and Muséum National d'Histoire Naturelle, *Patrimoines naturels* 29, Paris, France. [Reissue 2004.]).

Additionally, they do not represent riparian distributions well unless the grid quadrangles are quite small.

LANDFORM AND ELEVATIONAL SPOT MAPS

These maps are similar to the traditional spot map except that black or colored spots appear on shaded relief or elevational base maps. With use of a Geographical Information System (GIS), locations of man-made structures, geological formations, vegetation types, and substrate types can be added, if available. GIS technology is relatively new, so it has not yet been as widely used as some other map types (Fig. 10).

PROS This type of map can be produced in various sizes, with numerous overlay styles, and can be extensively and quickly modified. It definitely is the distribution map of the future.

CONS Production is of these maps is both expensive and time consuming, because all locality data must be converted to digital coordinates to ensure that dot placement on the map is accurate.

USE IN DIVERSITY STUDIES With the addition of landforms, distribution maps will begin to show the earth's surface as never before. Map size can be chosen to portray the area or areas of greatest interest and perturbations within that area over a certain time span. With sufficient locality information (the most difficult and time-consuming aspect of data gathering), both historic and dispersal proclivities of many taxa can be inferred. Maps can then become a valuable tool in diversity studies because they can be used to summarize what is happening over extensive areas of the earth's surface and through time.

Use of Maps in Diversity Studies

Certain of the map types described above can be used to depict baseline information on the actual distribution (both historic and contemporary) of a target organism. Museums and other scientific institutions maintain extensive collections of vouchers (see Chapter 6, "Voucher Specimens") of most reptiles and amphibians. Specimen accumulation started in the mid-1700s in Europe, with the naming and formal description of both plants and animals. Similar accumulations started in the New World in the early 1800s, so most of what we know about organismal distributions in the United States began at about that time. However, many of the earlier works had very general or even erroneous locality information, so many of those data are questionable and unusable today. Most of the more recent information (from the late 1800s to the present) is now accessible on the Internet, but this is not true for all institutions. One can contact the person in charge at each institution (usually a curator) and request the locality information needed. Institutions that maintain collections usually have special interest areas that they tend to emphasize; state collections, for example, tend to emphasize specimens from their own states. Researchers should contact these museums first if the target organism occurs in particular states. Most of the larger museums have world-wide coverage, but they also have favored areas, often reflecting the interests of members of the curatorial staff.

Once the locality records are in hand, the investigator can begin thinking about how to make his or her map or atlas. He or she first must decide whether or not to publish all the records. In 1985, when I started work on my *Interpretive Atlas of Texas Lizards* (e.g., Axtell 1988a, 1988b, 1992, 1996, 1999, 2000,

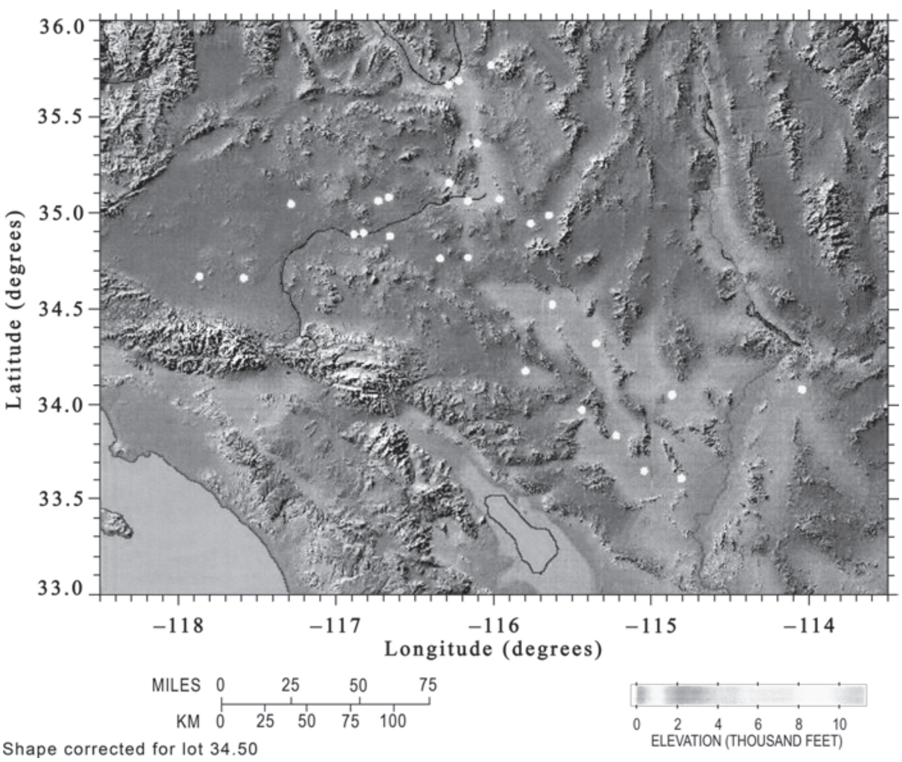

FIGURE 10 Landforms spot map of the distribution of the Mohave Fringe-Toed Lizard (*Uma scoparia*) in southern California and adjacent Arizona (localities transformed from Norris 1958; base map and plot courtesy of Dr. Ray Sterner, © 2010 Johns Hopkins University Applied Physics Laboratory; reprinted with permission). Note that distributional structure is present and that the direction of movement and possible movement routes can be inferred. More dots and the addition of sand deposits (with GIS) would make this map exemplary of what might be expected of such a representation.

2003a, 2003b), publication of the records seemed a natural extension of the endeavor; how else could the study be repeated (i.e., the scientific method)? I still believe that the decision was a good one, but others seem to think giving out detailed locality information is dangerous, for if it falls into the hands of "unscrupulous collectors," the target organism could suffer from "over-collecting." While this may be true under certain circumstances or with regard to particular species, I do not think it is true for a preponderance of Texas lizards. With one or two possible exceptions (i.e., Texas Horned Lizard [*Phrynosoma cornutum*] and Eastern Collared Lizard [*Crotaphytus collaris*]), there is little or no evidence to suggest that over-collecting has been a substantial problem. On the other hand, a benefit of locality listing is that dated collections and sites are readily available to those who may wish to monitor future population levels. In fact, I added dates to each locality in my lizard atlas after I realized that this information could be important for future investigators.

SPECIES INTRODUCTIONS

Possible species introductions can be identified if enough natural (native) localities are available for comparison. Introduced populations usually appear in small, isolated patches associated with villages and towns where people have released the animals. In preparing the Texas atlas, for example, I discovered that the Texas Horned Lizard had erratically populated much of eastern Texas (from the western border of Louisiana to near the lower Brazos River) mostly during the late 1950s to early 1970s (Fig. 11). All or almost all of these introduced populations disappeared for seemingly unknown reasons (although the disappearance coincided with the early stages of the introduced Red Imported Fire Ant [*Solenopsis invicta*] eradication program). The Texas Parks and Wildlife Department declared this lizard a protected species in 1977 (Andrew Price, pers. com.), apparently unaware that the area from which all those lizards disappeared was not part of the natural historic range of the species. Contemporary field guides at the time, however, had included eastern Texas as a part of the range of this horned lizard (such publications generally do not attempt to discriminate between natural and introduced ranges). Later it was discovered that the Texas Horned Lizard continued to exist in many parts of its natural range (see records in Axtell 1996) but no longer could be found in many agricultural areas where both pesticides and herbicides had been in common use. In Texas, the introduced fire ant continues in its relentless movement westward (W. P. MacKay, pers. comm.).

MOVEMENT PATTERNS

Movement (or dispersal) patterns can be proposed if adequate numbers of localities are available. Such patterns are most easily identified with movements along streams or waterways (riparian dispersal), but often, overland dispersal or special habitat dispersal are also recognizable. Dispersal always takes place when populations are high and expanding, but this may happen very rarely (perhaps once every 25 to 50 years or

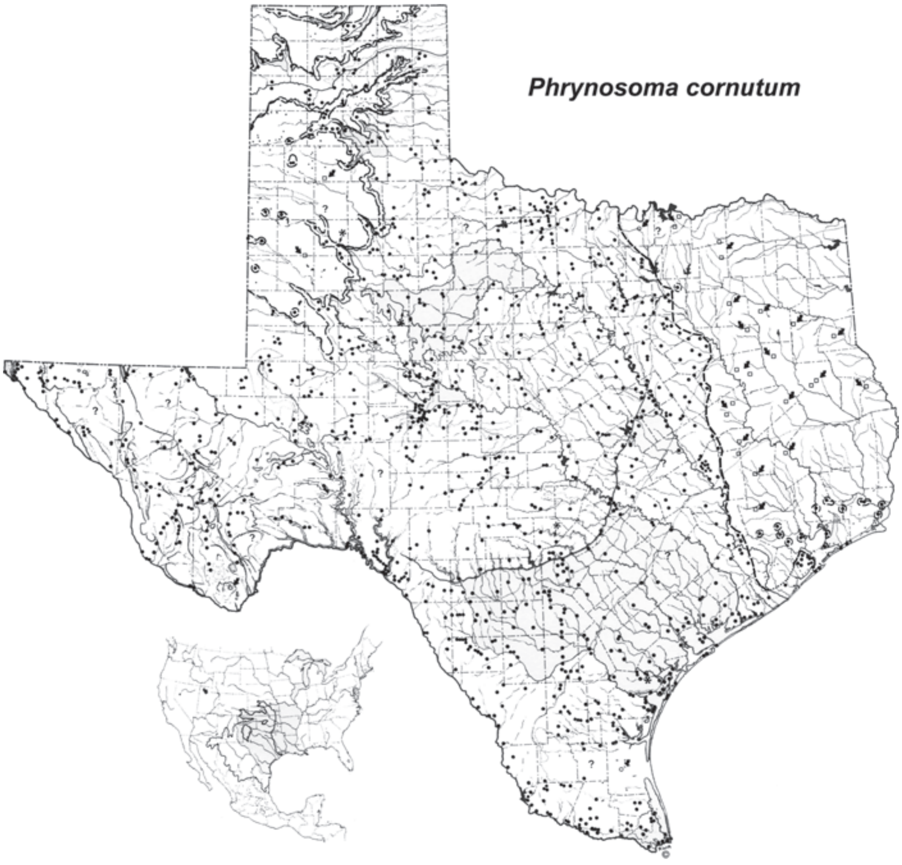

FIGURE 11 Distribution map of the Texas Horned Lizard (*Phrynosoma cornutum*) in Texas, showing localities of vouchers of naturally occurring individuals and localities of presumed introductions (in eastern Texas, marked with arrows). The dashed line denotes the interpreted natural eastern margin of the species range in the state. The encircled eastern coastal and High Plains localities are considered to be disjunct and may or may not represent introductions. Fossil localities are marked with asterisks. (Reprinted from Axtell 1996, map 9.)

more), so the patterns apparent on spot maps may be quite old (Fig. 12). Local dispersal takes place much more frequently, but such patterns usually do not show up on spot maps. Dispersal now and in the future will certainly be affected by all human enterprises (e.g., cities, highways, roads, factories, airports, ditches), so gene flow between many populations will be severely adversely affected. Planning remediation to mitigate such problems should begin as soon as possible.

DISJUNCTIONS

By their very nature, population disjunctions, or separations, tend to imply either a range retraction from a previously populated area or, more rarely, expansion into a new region. In the former case, intermediate populations should be largely absent, whereas in the later they should be obviously present. Disjunctions almost always are quite clear on spot maps (Fig. 13), but interpretations often differ as to whether or not these are actually disjunctions. On some maps sites are simply subjectively connected, implying that intermediate populations do exist. However, if no substantiating information is supplied, an interpretation favoring disjunction over connection is generally more appropriate, especially if the region has been well explored.

SPECIAL HABITATS

Although special habitats (i.e., sands, limestone or igneous rocks, certain types of vegetation) are not shown on most range maps, the inclusion of these features is now possible in many cases with GIS and with other well-prepared maps (Fig. 12). Some organisms are limited geographically by substrate or vegetation type. Inclusion of such habitat features on a map enhances the credibility of assertions made about the relationship.

RANGE OVERLAP AND JUXTAPOSITION OF RANGES

In the past, the ranges of almost all species were treated as discrete entities and mapped as such. However, many related taxa occur in close proximity and, if circumstances permit, may be able to interact genetically. Because of the tradition of depicting single species on a map, researchers may overlook interesting, and often illuminating, associations (e.g., see Axtell 1999; 2000, map 12 inset, for the association between *Plestiodon multivirgatus* and *P. septentrionalis* in eastern Nebraska). Because empirical evidence from the field of any kind of interaction between taxa is far superior to indirect evidence derived by other means, I recommend that we move away from the “one species–one map” tradition and instead

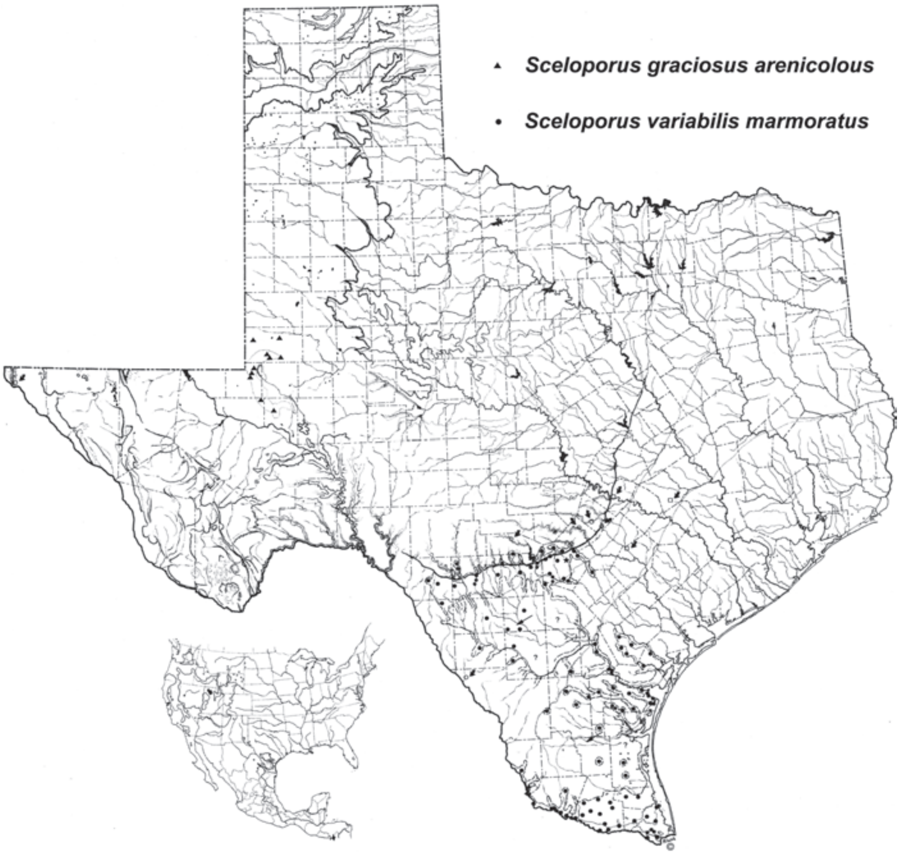
▲ Sceloporus graciosus arenicolous
• Sceloporus variabilis marmoratus

FIGURE 12 Distribution map of the Texas Rose-bellied Lizard (*Sceloporus variabilis marmoratus* [= *Sceloporus marmoratus* in Crother 2008]) in Texas, showing localities of vouchers of naturally occurring individuals (black dots), possible introductions or disjunct individuals (black arrows or circled dots), and one fossil locality (asterisk). Note the possible northern dispersal route from extreme southern Texas northward along the coast and then northwestward up the drainage systems of various river systems to near the Balcones Fault Zone (marked with a dark hatched line) in south-central Texas. Compare this interpretation with the county distribution map (Fig.8) from Dixon (2000: map 84). Note also in Fig. 12 the special habitat shown by the largely endemic distribution of the Dunes Sagebrush Lizard (*Sceloporus graciosus arenicolus* [= *S. arenicolus* in Crother et al. 2008]) in the Mescalero Sands deposits just opposite the southeastern corner of New Mexico. (Reprinted from Axtell 1988b, map 3.)

map related entities (whether species or subspecies) together, so that the areal relationship between them will be visually apparent.

RANGE CHANGES IN RECENT TIME

It is generally assumed that few distributional changes have taken place since the 1800s. This may generally be true for reptiles and amphibians; it is not true for certain mammals and birds. Both Nine-banded Armadillos (*Dasypus novemcinctus*; Taulman and Robbins 1996) and Cattle Egrets (*Bubulcus ibis*; Smith 1974) have invaded the United States from the south, moving hundreds of kilometers northward (for additional species, see Lomolino et al. 2006). Useful locality information began to accumulate in about the early 1900s, when good road maps first became available. Since that time, data on collecting localities have continued to improve, starting with the inclusion of distance and general direction (e.g., north, southwest) from a particular site (usually a town) and progressing through a particular coordinate (e.g., 5 mi N and 12 mi E of [some site]), then geographic coordinates (latitude and longitude), and most recently to digital readouts from a GPS.

DISTRIBUTIONAL AND PHYLOGENETIC AGE

Although not directly applicable in diversity studies, maps can be valuable adjuncts to phylogenetic inference by indirectly indicating phylogenetic age. In several groups that I have studied, including the "sand lizards" (*Uma, Callisaurus, Cophosaurus, Holbrookia*) and the collared lizards (*Crotaphytus* spp.), the oldest, or most "ancestral" taxa of the larger group tend to be found at or very near the periphery of the range of the entire group (genera or generic complexes in these cases). The prevalence of this phenomenon is presently unclear, but it contradicts Ernst Mayr's hypothesis that new taxa are concentrated at or near range peripheries (Mayr 1963, p. 513). It agrees instead with Brown's centrifugal speciation hypothesis (Brown 1957). Clearly, relationships exist between the ages of populations and their locations within species' ranges. Distri-

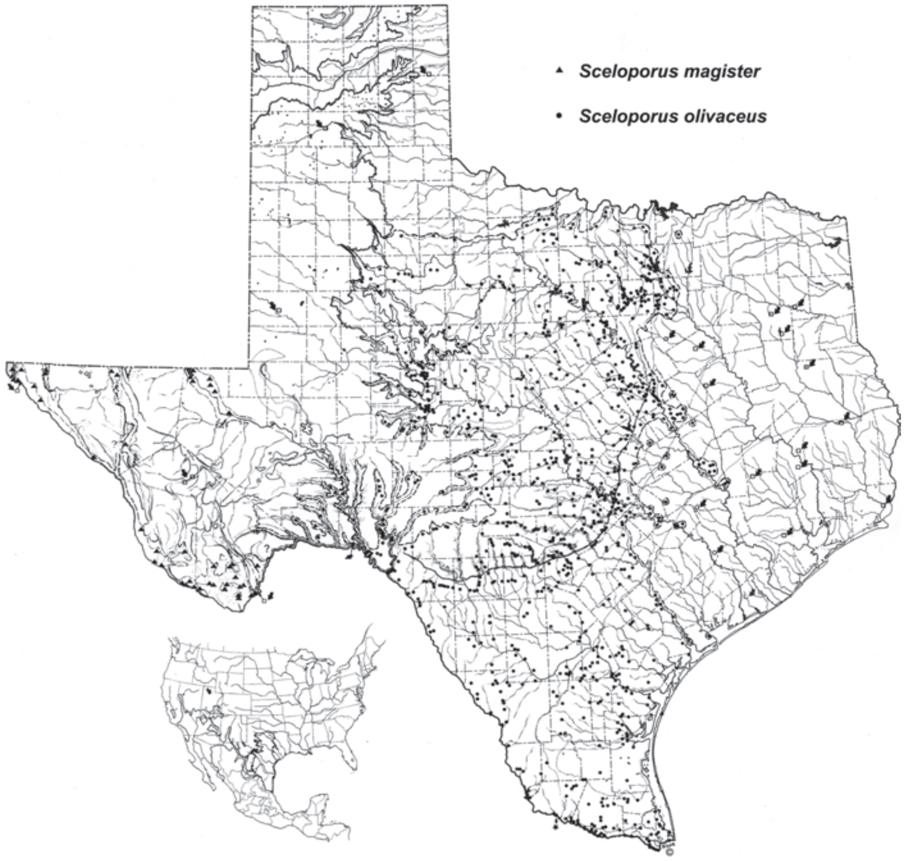

FIGURE 13 Distribution of the Twin-Spotted Spiny Lizard (*Sceloporus magister bimaculosus* [= *S. bimaculosus* in Crother et al. 2008]) in western Texas. Both the records and the range are considered disjunct. There is no evidence indicating that the Pecos Basin populations have ever crossed the narrow gap separating them from the Salt Basin populations directly to the west. Apparently, Texas Spiny Lizard (*Sceloporus olivaceous*) populations have recently moved westward, cutting off all access to the lower Pecos River and thus isolating the two *S. magister* populations. The locality of a single presumed hybrid between these two species is shown near the Pecos River mouth. (Reprinted from Axtell 1992, map 7.)

bution patterns of closely related taxa, especially ancient taxa, and the bases for those patterns remain to be determined.

An Atlas of Species Distributions

Whether maps encompass a single taxon or many, the eventual incorporation of those maps into an atlas is not of great importance to a general biodiversity study, and atlas production is very time consuming. However, bringing the distributions of large numbers of taxa together within a single project encourages comparison and analyses of the similarities and differences among them. Such considerations are likely to be extremely valuable when large areas are being evaluated for their ecological value and, consequently, their importance for preservation.

Acknowledgments

My thanks to Andrew Price, Texas Parks and Wildlife Department, Jake E. Schaefer, Department of Biological Sciences, University of Southern Mississippi, Ray Sterner, Department of Applied Physics, Johns Hopkins University, and Robert. G. Webb, Department of Biology, University of Texas at El Paso for their kind assistance with this project.

Automated Data Acquisition

Michael E. Dorcas and Charles R. Peterson

Data quantifying the relationship between changes in environmental variables (e.g., temperature) and changes in reptile activity levels and/or physiological states can be used to optimize sampling procedures for survey and monitoring programs and to interpret changes in reptile population levels (Peterson and Dorcas 1992, 1994). Our objective here is to describe the use of automated devices to measure variation in the physical environment as well as changes in the activity levels of reptiles.

We focus on continuous, automated sampling systems, rather than single, manual measurements, because such systems make it possible to measure a variety of variables accurately at one or more sites simultaneously. Being able to sample regularly over intervals of different lengths is important because many factors vary through time. Environmental attributes important to reptiles (e.g., temperature) can change dramatically over just a few hours, as well as over days, months, or seasons (Peterson et al. 1993). We will discuss how to monitor reptiles' environments automatically using dataloggers, environmental sensors, and physical models. We will also consider ways to (1) monitor reptile activity using automated radiotelemetry, (2) monitor body temperatures of reptiles with attached or implanted dataloggers, (3) monitor global positioning systems (GPS) attached to animals, (4) automate passive integrated transponder (PIT tag) systems, and (5) employ automated photographic and video systems.

We obtained some of the information we report concerning dataloggers and environmental sensors from Pearcy et al. (1989), Campbell (1990), and Tanner (1990), which readers may wish to consult. Throughout the text we cite the names of manufacturers or retailers, especially for equipment and materials that we have used (see Appendix II for vendor information). However, our experience with different techniques and different types and brands of equipment is limited; thus, the listing or omission of a particular vendor does not indicate either our endorsement or rejection. Furthermore, equipment is evolving rapidly, and many of our specific comments are already out of date.

Dataloggers

A datalogger is an instrument that records and stores data collected by one or more sensors. Dataloggers are generally programmed by some sort of computer (e.g., laptop). They operate autonomously, and the data they collect are downloaded to a computer after some prescribed period. All dataloggers work essentially the same way, by measuring voltages produced by sensors (i.e., transducers such as thermistors). Most dataloggers are able to convert raw voltages into the unit of interest (e.g., volts into degrees). Some dataloggers can also be used to control instruments, turning a piece of equipment on and off at specified intervals. Dataloggers are either single- or multichanneled. Single-channel dataloggers receive input from only one sensor, and multichannel dataloggers receive data from two or more sensors.

MULTICHANNEL DATALOGGERS

Within the past 30 years, the task of gathering continuous data has been greatly facilitated by the development of dataloggers suitable for use in the field. The distinguishing characteristics of field dataloggers include portability, battery power, programmability, and the ability to read inputs from several types of sensors at user selected intervals (Campbell 1990). Dataloggers have numerous advantages over devices such as mechanical recorders and strip chart recorders, including a wider operating temperature range, increased sensor compatibility, higher accuracy, greater data storage capacity, and much easier transfer of data to computers for analysis (Pearcy et al. 1989).

Factors to consider when selecting a mutlichannel datalogger include cost, reliability, the range of operating conditions (temperature and humidity), accuracy, resolution, number of channels, sensor compatibility, processing power, data storage and retrieval options, and power requirements (Tanner 1990). Costs range from approximately US$60 to more than US$5,000. Powerful, versatile systems, capable of reading most sensors, are available for less than US$1,700 (including the interface and software for downloading data). Features to look for in this price range include 12-bit or greater resolution, the ability to measure microvolts (e.g., thermocouples), switch or pulse-counting capability (for cup anemometers and tipping-bucket rain gauges), the ability to provide excitation voltages (for thermistors and electrical resistance humidity sensors), and digital outputs for controlling devices such as tape recorders and radiotelemetry systems. A more expensive datalogger capable of resolving nanovolts is required for measurements of some variables (e.g., soil-water potentials using thermocouple psychrometers).

If funds for purchasing a datalogger are not available, investigators should try to borrow one or to use data already being collected with a datalogger at or near their study sites. It may even be possible to add sensors to a multichannel system presently operating at the site. Costs of dataloggers are declining, and many universities, field stations, government agencies, parks, and similar institutions have machines available for use by researchers. Investigators should learn how to deploy and program their dataloggers. Becoming comfortable with the more powerful and flexible systems (e.g., Campbell Scientific) may require considerable time (i.e., at least several days or weeks). Investigators can minimize learning time by working with someone already familiar with the equipment. Some manufacturers offer training sessions that may be helpful for less technologically savvy users. Most dataloggers now come with user-friendly software that allows even the novice to begin using the equipment after minimal learning time. Onset Computer Corporation manufactures a relatively inexpensive weather station that is considerably easier to use than the systems produced by Campbell Scientific. In general, an entire Onset weather station (up to 15 sensors) can be set up in an afternoon by a person with moderate technical and computer skills. Onset's weather station runs for up to 1 year on 4 AA batteries and will hold 500,000 measurements in memory. Onset uses "smart sensor" technology in which the datalogger detects the type of sensor that is connected and then automatically calculates the proper output units from the sensor data. MED used an Onset weather station on an ecological preserve in North Carolina for 6 years with only minor problems in its operation (Fig. 14).

Dataloggers should be placed in some type of enclosed structure to protect them from the weather and vandals. Most manufacturers offer such containers, although small plastic toolboxes or the waterproof storage boxes used on boats are less expensive alternatives. In areas exposed to direct sunlight, it may be necessary to shade the enclosure or paint it white to prevent overheating. In humid environments, a desiccant (e.g., silica gel) can be placed within the enclosure to maintain operating conditions of the datalogger. Burial also reduces the range of temperatures to which the datalogger is exposed. In areas prone to flooding, dataloggers can be suspended from trees with ropes. To prevent vandalism and theft, we have sometimes buried enclosures to hide them.

Another frequent problem is animal damage to sensors or sensor wires. Because thermocouples or thermistors often must be placed at considerable distances from the datalogger, they are particularly vulnerable. Moose (*Alces alces*) and other large mammals have damaged thermocouples or pulled them loose from our dataloggers, and rodents have chewed through the insulation on connecting wires. Burying or covering the exposed parts of the wires with moderate sized rocks or logs may help to prevent such damage, as does enclosing thermistor wires in small diameter PVC pipe.

SINGLE-CHANNEL DATALOGGERS

Single-channel dataloggers have several advantages relative to multichannel systems. They are much simpler and less expensive and perfectly adequate when only one or a few environmental variables need to be measured. Additionally, single-channel dataloggers are ideal if environmental variables must be measured at locations some distance apart. Also, because of their small size, single-channel loggers are often much easier to deploy, protect from environmental conditions, and hide from potential vandals. Specially designed single-channel dataloggers that measure temperature, solar and lunar radiation, barometric pressure, relative humidity, precipitation, soil moisture, or wind speed are available.

We have had experience using two types of single-channel dataloggers. For most of our applications requiring monitoring of one or a few variables, we have used dataloggers from Onset Computer Corporation (US$60–$400). These single-channel dataloggers are easily programmed and their stored data easily downloaded through a serial or USB interface using the Hoboware software available from Onset. The software runs on Windows and Macintosh operating systems, is easy to use, automatically recognizes the type of datalogger attached, and allows downloaded data to be easily exported in text or Microsoft Excel formats for analysis.

We have also used the iButton Thermochron datalogger, developed by Dallas Semiconductor, which provides a considerably smaller alternative to many of the single-channel dataloggers when temperature measurements are required. Thermochrons are 5.9 mm high by 17.4 mm in diameter (approximately the size of four stacked dimes) and weigh 3.12 g. In addition, Thermochrons are relatively inexpensive (approximately US$20–$85 each). Thermochrons are reliable and accurate in laboratory calibrations (Angilletta and Krochmal 2003) and are programmed through a serial or USB computer interface. Thermochrons have a somewhat limited capacity, however, recording from 2,048 to 8,192 time- and date-stamped temperature readings with 0.125 to 0.5°C resolution (depending on model), at sampling intervals selected by the user (Angilletta and Krochmal 2003; Robert and Thompson 2003; Grayson and Dorcas 2004; Harden et al. 2007; Harden and Dorcas 2008).

Both Onset Computer and Dallas Semiconductor provide software and interface cables that allow downloading and limited programming of their dataloggers using personal digital assistants (PDA's) running the Palm operating system. We have used PDAs to download data from Onset dataloggers in the field and found them to be very reliable. After downloading, the PDA is connected to a computer, and the downloaded data are transferred to it. We have also used tablet PC's to interface with our dataloggers in the field. Although a field computer (i.e., laptop) is larger than a PDA, we find the flexibility provided by the standard Microsoft Windows operating system makes transfer of data less complicated and reduces time spent learning or teaching someone how to use PDA-based software. It should be possible to connect and use PDA's running the Microsoft mobile operating system to program and download data from most dataloggers that require connection to a MS Windows environment, although we have no experience with this procedure.

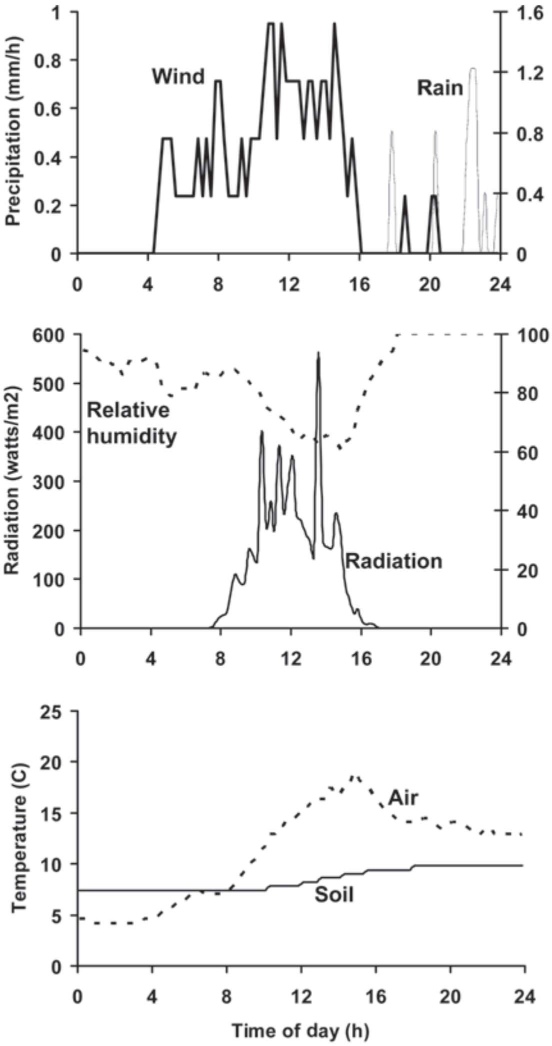

FIGURE 14 Variation in wind, precipitation, relative humidity, solar radiation, air, and 20-cm deep soil temperatures sampled using a weather station (Onset Computer Corp.) every 30 minutes for 24 hours (4 December 2005) at the Davidson College Ecological Preserve.

Environmental Sensors

In the following sections we describe the sensors that are most often used with dataloggers to measure important environmental variables. For weather stations, sensors are usually mounted on an instrument tripod that can be purchased from a supplier or constructed by the investigator. Examples of measurements of air and soil temperatures, radiation, relative humidity, and wind speed are plotted versus hour of day in Figure 14. We do not have extensive experience with measuring all of these variables and thus have had to rely on the literature or advice from engineers or other scientists to prepare some of the following material. See Fritschen and Gay (1979), WMO (1983), Marshall and Woodward (1985), Finklestein et al. (1986), Pearcy et al. (1989), Skaar et al. (1989), Campbell (1990), and Tanner (1990) for more information on sensor instrumentation.

TEMPERATURE

For a variety of reasons, thermocouples are sometimes the preferred sensors to use in field studies requiring automated temperature measurements. They are relatively accurate, inexpensive, come in a wide range of sizes, and respond quickly, and their signal does not diminish over long distance, which may be especially important when using multichannel dataloggers (Pearcy et al. 1989). For temperature measurements in the range of biological interest (–70 to 100°C), Type T (copper-constantan) thermocouples are most appropriate because this combination of metals produces a relatively large voltage that changes linearly with temperature (ca. 40 μv/°C) within the range of interest. Thermocouples come in a wide range of sizes; 24-gauge (0.5 mm diameter) wire is commonly used for measuring water, soil, and air temperatures. Thermocouple wire is relatively cheap (about US$1.75/m) depending on the type, size, and quality of the wire. Thermocouples can be purchased or easily made by stripping the ends of the wire, twisting the exposed ends together, and then soldering them (Pearcy et al. 1989). The actual site of the temperature measured lies at the first junction of the twisted wires (i.e., the thermocouple). Thermocouples usually do not have to be individually calibrated. Potential problems with thermocouples include heat conduction via the thermocouple wire and inaccurate measurements of the reference temperature (usually taken where the thermocouple wire attaches to the datalogger).

Thermistors (temperature sensitive resistors) are another device commonly used to measure temperature. They are highly sensitive and accurate, and they respond rapidly. However, thermistors are more expensive and more delicate than thermocouples, cannot easily be used over long distances, and sometimes require individual calibration. Some dataloggers can read both thermocouples and thermistors easily, whereas other dataloggers read only one type, or read a second type only with difficulty.

Most single-channel temperature dataloggers use thermistors as the temperature sensor. However, some single-channel dataloggers are able to read thermocouples. Some single-channel dataloggers come with the sensor built into the logger, and thus temperature fluctuation of the entire datalogger (or a portion of it) is actually measured. Others provide the option of plugging in a thermistor or thermocouple for measurement some distance away from the datalogger. Many single-channel dataloggers designed to measure temperature do not require any special housing and can simply be mounted or positioned where desired. Specially designed, waterproof, single-channel dataloggers for measuring water temperature (e.g., Stowaway-Tidbits, Onset Computer) are available and can be tethered and tossed into the water. Other dataloggers may be used to monitor water temperature, but they may require a waterproof housing with the temperature sensor (e.g., thermistor) exiting the housing through a sealed port. We have successfully used wide-mouthed plastic bottles (i.e., Nalgene) for this purpose.

We usually measure air temperatures at animal height (e.g., 1 cm) and/or at 2 m (a standard reference height for meteorological stations), water temperatures on the bottom and 1 cm from the upper surface, and soil temperatures from at least two depths (e.g., 1 and 20 cm) as surrogates for burrow temperatures. Thermocouples and thermistors used to measure air temperatures should be shaded unless they are small and highly reflective (e.g., painted white). Some manufacturers provide radiation shields for temperature dataloggers and sensors at a moderate cost. To measure shallow water temperature in aquatic environments where the water depth may fluctuate greatly over short periods (hours or days), we attach the end of the thermistor or thermocouple to a float (e.g., a bobber used for fishing) that is attached loosely to a metal rod anchored in the bottom so that the sensor can rise and fall with fluctuating water levels.

PHYSICAL MODELS AND OPERATIVE TEMPERATURES

Temperature is one of the most important factors influencing the activity of reptiles (especially in the temperate zone) and, thus, our ability to determine their presence and abundance (Peterson et al. 1993). For this reason, it is important to describe the thermal environments of reptiles accurately. The thermal environment of submerged, aquatic reptiles (e.g., some turtles) can be characterized relatively easily by measuring the temperature of the surrounding water. Describing the thermal environments of terrestrial reptiles is more complex, because a variety of factors interact to determine their body temperatures. These factors include air temperature, substrate temperature, radiation, humidity, soil moisture, wind speed, and animal properties such as size, shape, and reflectivity (Bakken 1992). A single-number representation of the thermal environment that incorporates these factors is the *operative temperature* (Bakken and Gates 1975; Bakken 1992). Operative temperatures can be calculated using computer models of heat transfer, but this approach may be difficult to apply at small spatial scales and requires considerable instrumentation and expertise.

A simpler and less expensive approach for measuring operative temperatures involves the use of physical models that incorporate animal properties such as size, shape, and reflectivity (Bakken and Gates 1975). This approach has been applied with considerable success to dry-skinned ectothermic vertebrates (i.e., reptiles; see Crawford et al. 1983, Peterson 1987, and Grant and Dunham 1988). Many investigators use hollow casts of actual reptiles as models. A convenient alternative is a section of hollow copper tubing. We found that the temperature of a model made of copper tubing of approximately the same length and diameter as a real snake and painted to match the reflectance of the dorsal surface of that species of snake generally closely matches the temperature of the real snake (Peterson et al. 1993). Temperatures of models of small reptiles tend to match those of the real animals closely. In contrast, the thermal inertia of large reptiles usually results in substantial lag before the actual body temperatures of these animals reach those of hollow models. Vitt and Sartorius (1999) found that self-contained dataloggers such as Tidbits (Onset) may serve as good estimators of body temperature for some small reptiles (e.g., many lizard species). Shine and Kerney (2001) provided a detailed analysis of various physical parameters that may affect temperatures of physical models.

HUMIDITY

Electrical resistance sensors and capacitance sensors provide convenient ways to measure atmospheric water vapor automatically (Tanner 1990). Costs range from US$85 to US$400. Some of these sensors require that the datalogger provide an AC excitation voltage. Sensor elements need to be individually calibrated at least annually and may need to be replaced periodically. Some electrical humidity sensors may be damaged by condensation or air contaminants (Campbell 1990). Among commercial hygrometers, ventilated wet-bulb, dry-bulb psychrometers are the most accurate but also more expensive, require power to run the fan, require attention to keep the water reservoir filled, and do not read accurately below freezing. See Skaar et al. (1989) for a comparison of commercial hygrometers.

Onset Computer Corporation provides a dual-channel datalogger (HOBO Pro RH/Temp) that measures atmospheric water vapor and air temperature. The Hoboware software can calculate and output temperature, relative humidity, and dew point. Cost is approximately US$200 with the recommended rain shield.

PRECIPITATION

Precipitation can be measured automatically with a tipping-bucket rain gauge connected to a pulse-counting channel on a datalogger (Tanner 1990). When a specified depth of water has collected, the bucket tips and empties. The datalogger records the number of tips. Resolution in the range of 0.1 to 0.2 mm is possible (WMO 1983). Onset single-channel event recorders can be used to log data from a single tipping-bucket rain gauge (cost is about US$400). Tipping buckets can be heated so that winter precipitation (snow, ice) will melt and the water will drain from the bucket. Weighing-bucket rain gauges provide a more accurate but more expensive way to measure precipitation (Tanner 1990).

RADIATION

Two types of solar radiation sensors (*pyranometers*) that are commonly used with dataloggers are silicon photocells (e.g., Li-Cor LI2000SB) and thermopile devices (e.g., Eppley Model PSP, Kipp and Zonen Model CM11). Silicon cells are considerably less expensive than the thermopile devices (approximately US$200 vs. $1300–$3000). However, because the spectral response of silicon cells is limited (400–1100 nm), they should not be used under vegetation canopies or to measure reflected radiation (Tanner 1990).

Lunar radiation can also affect the levels of activity of some reptiles (Clarke et al. 1996). Single-channel radiometers that measure solar and lunar radiation are available from Onset Computer. We successfully used one of these (Stowaway LI) to measure solar and lunar radiation as part of a study examining factors affecting snake activity in South Carolina (J. Willson, unpubl. data). Light intensity is recorded on a logarithmic scale (log Lumens/m^2), allowing easy visualization of lunar and solar radiation (Fig. 15).

WIND SPEED

Wind speed can be measured automatically with a cup anemometer and the pulse-counting channel of a datalogger. Cup anemometers are omnidirectional, have linear responses, and are reasonably precise (Campbell 1990). Factors to consider when selecting an anemometer include size, the range of wind speeds over which the sensor operates (especially the starting and stopping thresholds), cost, and durability. Propeller anemometers have lower thresholds and can measure wind direction, but they are more expensive (Campbell 1990). We usually mount anemometers at a height of 2 m. If more than one anemometer is available, wind profiles can be determined so that the 2 m wind speed can be used to calculate wind speeds at other heights.

Automated Monitoring of Animals

Monitoring animal activity and correlating that activity with variation in environmental variables allow an investigator to predict when animals are likely to be most active and thus most easily encountered for inventory or monitoring purposes (Peterson and Dorcas 1992). Additionally, some automated systems can provide information on animal locations, so habitats most often used by animals can be identified and sampling efforts further refined. Some types of reptiles are more easily monitored with automated devices than other types. For example, satellite GPS may be useful for monitoring large chelonians such as sea turtles, but it is inappropriate work for smaller species because of the size of the equipment. Below, we review a variety of automated methods that have been used to monitor reptile populations or that have been used to monitor other animal groups but can be adapted for some species of reptiles.

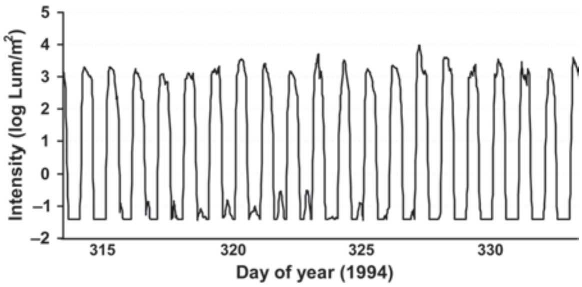

FIGURE 15 Variation in light intensity (solar and lunar radiation) over a 20-day period in November 1994 at Ellenton Bay on the Savannah River Site, South Carolina. Note the progressive delay in moon rise from day 316 until day 327 and the absence of measurable lunar radiation after day 328.

AUTOMATED RADIOTELEMETRY

Radiotelemetry has been used extensively to monitor the movements and habitat use of many animals, including many species of reptiles (Millspaugh and Marzluff 2001). Automated systems used in conjunction with radiotelemetric techniques can also be used to monitor body temperature automatically and continuously in many species of reptiles. Body temperature variation, especially when combined with environmental temperatures, can then be used to make accurate inferences about activity patterns and microhabitat use (Fig. 16).

Generally, the factor determining whether reptilian species are suitable for radiotelemetric projects is size; large animals are more easily outfitted with radiotransmitters than small ones. However, the continued development and refinement of telemetry equipment is making it possible to use automated telemetry techniques to monitor smaller and smaller species. For example, we used an automated system with a small, surgically implanted transmitter (ca. 2 g; BD-2, Holohil Ltd.) to monitor the body temperature of a 35-g, male Northern Rubber Boa (*Charina bottae*) for 3 months. The snake fared well, gaining 15 g during the study period.

For snakes, most transmitters must be surgically implanted; thus, temperature-sensitive transmitters monitor body temperatures accurately and automatically (Peterson et al. 1993). These transmitters use a temperature-sensitive resistor (thermistor) that causes the pulse rate of the transmitter to vary positively with temperature. The addition of a temperature-sensitive option to a typical transmitter generally adds about US$50 to $75 to the cost, but it does not substantially increase the size

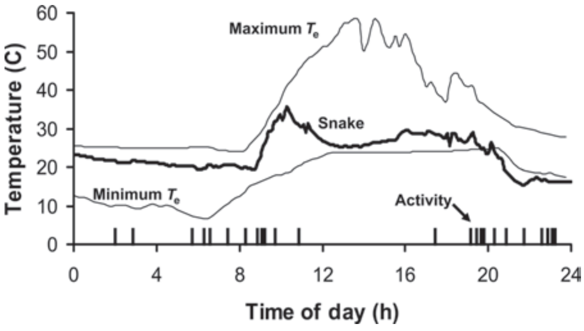

FIGURE 16 Variation in the body temperature and activity of a Great Basin Rattlesnake (*Crotalus oreganus lutosus*) in southeastern Idaho over 24 hours in the summer of 1991. Temperature was monitored using a thermistor within a surgically implanted radiotransmitter. The thermistor caused the pulse rate of the transmitter to vary with temperature. Activity was determined by toggles of a mercury switch within the transmitter. Transmitter pulse rate was monitored with an automated system described in the text. Environmental temperatures (T_e) were determined with physical snake models and a Campbell datalogger (Cobb 1994).

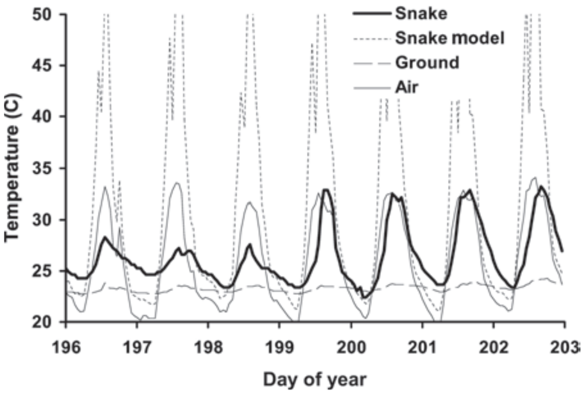

FIGURE 17 Variation in body temperature of an Eastern Diamondback Rattlesnake (*Crotalus adamanteus*) over a 1-week period in August 1999 in southeastern South Carolina. Body temperature was measured using a surgically-implanted datalogger (see text). Environmental temperatures were measured using thermocouples and a multi-channel datalogger (CR-10, Campbell Scientific). Apparently, the snake was in a shallow burrow the first 3 days and then remained on the surface in the shade, for the rest of the week.

or mass of the transmitter. Temperature-sensitive transmitters should be calibrated in a water bath before surgical implantation, and equations that describe the relationship between temperature and interpulse interval should be developed (we typically use a second- or third-order polynomial). Some manufacturers calibrate temperature-sensitive transmitters for the researcher, but we recommend that she or he verify those calibrations. Also, due to the unfortunate experiences of some of our colleagues, we highly recommend that transmitters be calibrated after removal to check for a shift in the temperature sensitivity.

A variety of automated systems have been developed to monitor animals outfitted with radiotransmitters. Automated systems that can monitor temperature-sensitive radiotransmitters usually are designed to record the rate of the interpulse interval. The simplest of these systems monitors only one animal (i.e., one frequency) and has a recycling timer that automatically turns a radio receiver that is tuned to the transmitter frequency and a cassette tape recorder that records the pulse rate as an audible signal on and off at regular intervals (Beaupre and Beaupre 1994; Cobb 1994; Lutterschmidt et al. 1996). The tape must then be manually decoded (i.e., the pulse intervals timed) using a stop watch or other timer.

More advanced systems can scan systematically over many frequencies (i.e., monitor multiple animals), time the interpulse intervals, and then store the data, which can later be downloaded into a computer. We have used a Fast Data System (Telonics; see Appendix II) for this purpose to study body temperature variation in free-ranging Terrestrial Gartersnakes (*Thamnophis elegans*; Peterson 1987) and Northern Rubber Boas (Dorcas and Peterson 1998). More advanced systems have been developed that use dataloggers to turn the receivers on and off, change receiver frequencies, and time interpulse intervals (Grothe 1992; Cobb 1994). LoTek has developed an elaborate but expensive (ca. US$14,000) system that allows the user to enter calibration values so that interpulse intervals are automatically converted into body temperatures (S. J. Beaupre, pers. comm.).

Some manufacturers can outfit their transmitters with activity switches (usually mercury switches) that allow detection of animal movements (Grothe 1992; Cobb 1994). When combined with temperature-sensitive capabilities and automatically monitored, these units allow for strong inferences regarding activity patterns and microhabitat use (Fig. 16). Because the amount of movement necessary to trigger the switch depends on the position of the sensor in the animal, we recommend only intra-individual comparisons. Additionally, these activity transmitters should be calibrated to determine how much movement is required to trigger the sensor.

ANIMAL TEMPERATURE MONITORING USING DATALOGGERS

Automated monitoring of body temperature is possible with temperature dataloggers attached to (or implanted in) reptiles. MED used Onset Tidbit dataloggers to monitor the body temperatures of Eastern Diamond-backed Rattlesnakes (*Crotalus adamanteus*) automatically, while tracking their movements using radiotelemetry. He used Tidbits, which he programmed, coated in polymer (Elvax by Minimitter), and then implanted into the body cavity. By examining body temperature plots and with environmental data, he was able to infer activity patterns and microhabitat use (Fig. 17).

We have successfully used Thermochron iButtons to measure seasonal variation in body temperature and infer activity patterns (e.g., basking) in Painted Turtles (*Chrysemys picta*) inhabiting a farm pond (Fig. 18; Grayson and Dorcas 2004). We glued the Thermochrons to plastic cable ties and attached them through holes drilled in the marginal scutes of the carapace. After programming, Thermochrons were sealed with a fast-drying liquid plastic tool dip (Plasti Dip International) traditionally used to coat the grips of hand tools. We recovered 41 Thermochrons from the turtles and upon recovery were able to download data from all of them successfully. We conducted similar studies with Diamond-backed Terrapins (*Malaclemys terrapin*) and Eastern Mud Turtles (*Kinosternon subrubrum*), easily distinguishing periods of basking, microhabitat use, and aquatic and terrestrial activity (Harden et al. 2007; Harden and Dorcas 2008).

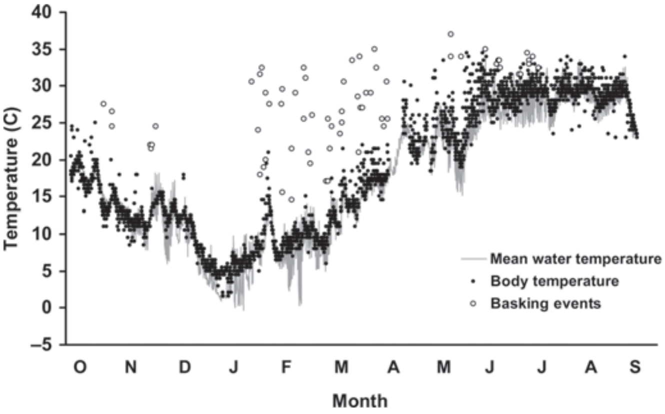

FIGURE 18 Seasonal temperature variation of a male Painted Turtle (*Chrysemys picta*) monitored for 1 year using a Thermochron iButton (Grayson and Dorcas 2004). Water temperatures were measured using Onset Tidbit dataloggers. Basking temperatures were defined as air temperatures 6°C greater than the water temperature.

AUTOMATED MONITORING OF PIT TAGS

Passive integrated transponders (or PIT tags) have become a widely used method of individually marking a variety of animals (Gibbons and Andrews 2004). Various automated systems for monitoring animals implanted with PIT tags have been developed (e.g., for fish, Prentice et al. 1990; voles, Harper and Batzli 1996; and bats Kunz 2001). Generally, animals implanted with PIT tags or with PIT tags attached to them are monitored as they pass through an opening or over a specific area where a PIT tag reader is placed. Boarman, Beigel, et al. (1998) developed an automated system using Psion dataloggers to read PIT tags and monitor the movements of Desert Tortoises (*Gopherus agassizii*) when diverted under highways through culverts. Each time a tortoise passed over the reader's detecting coil, the system recorded its PIT-tag identification number, time of day, date, and period during which the tag was within reading distance of the coil. Gruber (2004) used an automated PIT-tag reading system to monitor the activity of geckos (*Gehyra variegata*). He used a datalogger connected to a PIT tag–reading coil that encircled the base of a tree to detect the passage of geckos to which PIT tags had been glued.

Pit tags may prove ideal for monitoring the activities of reptiles in certain other situations. The hibernacula of some snakes, especially in northern latitudes, may have discrete openings through which the movements of snakes implanted with PIT tags could be automatically monitored relatively easily. PIT-tagged reptiles passing through openings in drift fences could also be monitored using automated PIT-tag readers (Gibbons and Semlitsch 1982).

Temperature-sensitive PIT tags have been used to manually monitor thermoregulation in Red Cornsnakes (*Pantherophis guttatus*; Roark and Dorcas 2000) and Northern Rubber Boas (Zhang et al. 2008). PIT-tag readers that can monitor temperatures and read such PIT tags (BioThermal TX1440BBT) from a short distance (e.g., 25 cm; BioMark) may allow automated monitoring of reptiles implanted with temperature-sensitive PIT tags.

AUTOMATED GLOBAL POSITIONING SYSTEMS

Elaborate automated systems for tracking animals with attached global positioning systems (GPS) have been developed for many species of large mammals and some birds (Rogers 2001). Several types of systems can be used to transmit location data from the GPS to the investigator. Some systems use radio signals, more sophisticated systems use satellite links (Argos satellite system), and others store data on board the GPS unit until it is retrieved. For many investigators, however, the costs of such systems are prohibitive. Units that transmit data via radio signals or satellite links range from approximately US$3,500 to $5,000 each. Systems that store data are considerably cheaper (ca. US$2,000–$2,500).

Historically, only large reptiles have been monitored with GPS because of the cost and weight of the systems. Consequently, automated GPS use with reptiles has been limited primarily to studies of sea turtles and the very largest freshwater turtles. However, smaller systems designed for birds that weigh less than 20 g (TAV-2417 Argos, Telonics) are now available and could be used on (or implanted into) some reptile species. Satellite GPS systems have been successfully used to monitor the movements of immature Loggerhead Sea Turtles (*Caretta caretta*; Cardona et al. 2005). Even fine-scale movements can be monitored, allowing precise data on location and depth to be collected (Yasuda and Arai 2005). Satellite transmitters have also been used successfully to monitor the movements of South American River Turtles (*Podocnemis expansa*) in Brazil (R. Vogt, pers. comm.). Balazs et al. (1996) provided detailed instructions for attaching satellite GPS transmitters to the carapaces of turtles, and similar techniques could be applied to other reptilian taxa. If funding were sufficient, automated GPS systems could likely be used with medium- to large-size reptiles and possibly even with some crocodilians.

ANIMAL MONITORING WITH PHOTOGRAPHY

Automatically controlled cameras provide unique opportunities for the remote observation of animal activities, providing information useful for optimizing inventory and monitoring programs. Such systems may also be used to document the occurrence of rare species unlikely to be observed directly. Several investigators have used cameras that take a still image when a light beam is broken or when a switch is mechanically triggered. Tuberville and Burke (1994), for example, used 35mm film cameras that were activated by the interruption of a light beam to detect potential predators of eggs in freshwater-turtle nests. DeVault and Rhodes (2002) used 35-mm film cameras triggered by a mechanical switch to investigate scavengers. Their camera, in a weather-resistant case attached to a tree, was triggered when a dead rat, which was holding down the switch, was moved. It took an image of the area surrounding the dead rat. Although not reported in their published paper, which focused on mammal scavengers, several snakes were discovered scavenging the dead rats used as bait (T. L. DeVault, pers. comm.). Guyer et al. (1997) described in detail the construction of a system that uses a modified digital camera (Canon SureShot Max/Date) in conjunction with a pressure switch plate to monitor vertebrate activity. The use of a digital camera for applications such as this greatly increases the image capacity of the system and, because film developing is not required, greatly reduces costs. Campbell Scientific (see Appendix II) produces a digital camera (model CC640) for use in harsh environments that can easily be triggered by timing devices or by dataloggers.

Numerous investigators have used automatically controlled still and video cameras to record predation (including by snakes) at bird nests (e.g., Renfrew and Ribic 2003; Peterson et al. 2004) and other activities of reptiles. Some researchers have used time-lapse video (2–5 frames/s), which allows for longer recording times than if the camera were allowed to run continually. However, triggering a video camera to record based on stimuli such as a switch or interruption of a light beam is possible and may allow for longer periods without maintenance because recordings are only made when the switch is triggered. Furman Diversified (Appendix II) produces a wide range of video-camera systems (analog and digital) specifically designed for use in the field. However, many researchers are now using various of the relatively inexpensive commercially available motion-sensitive still and video cameras (e.g., StealthCam; see Appendix II) used for hunting and wildlife observation. Such devices are easy to use, and the relatively low price of many of these units makes them affordable to many researchers. However, because many reptiles are small or move slowly, the movement required to trigger many of these cameras can make them impractical for some reptile studies. For example, J. D. Willson and J. W. Gibbons (pers. comm.) used a motion-sensitive video camera to monitor consumption of a road-killed opossum by a large (3-m) Burmese Python (*Python molurus bivittatus*) kept in an outdoor enclosure in South Carolina. Despite the fact that the camera was positioned only 2m from the dead opossum, the snake apparently moved so slowly that the camera was not triggered, and the python consuming the opossum was not recorded.

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Handheld Computers for Digital Data Collection

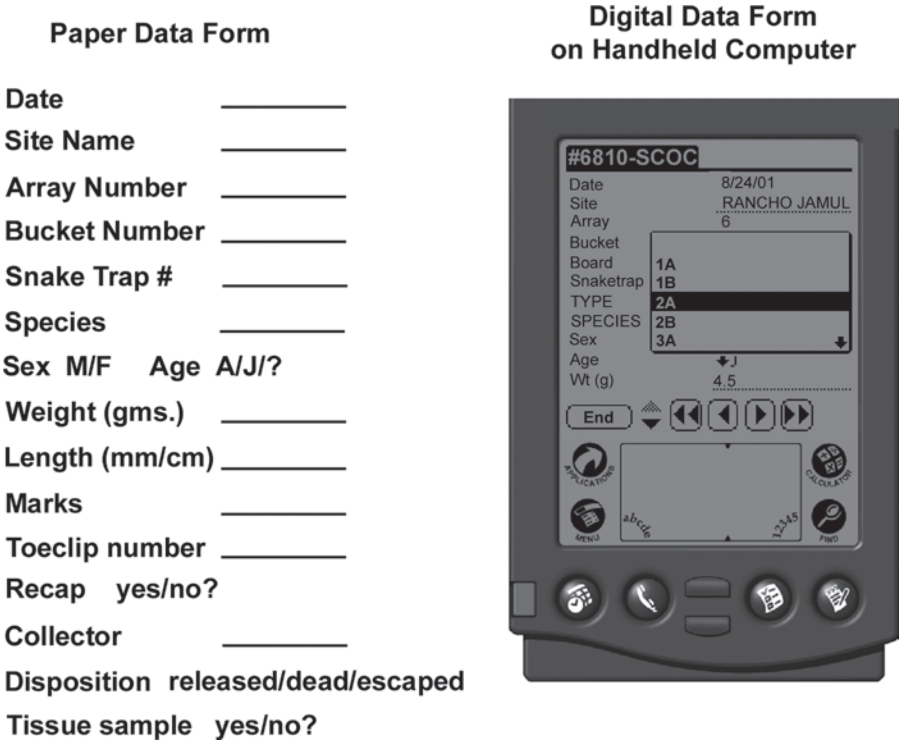
Carlton J. Rochester and Robert N. Fisher

Many publications outline the types of data to collect and data fields to use when carrying out biodiversity surveys; such articles often suggest designs for paper data forms as well (Heyer et al. 1994a). Here, we describe an alternative to traditional pen-and-paper data recording. With recent advancements in handheld computers and their software applications, the development and use of electronic forms for recording data (using Personal Digital Assistants, or PDAs), a topic that has only been mentioned briefly in previous literature (e.g., Donnelly and Guyer 1994), has become a reality. Multiple operating platforms for PDAs, including handheld PCs, Pocket PCs, and the Palm OS (see Appendix II), are now on the market. Each one offers mobile, electronic data collection and its associated benefits to investigators in the field. Below, we outline some of the advancements that have been made over the past 10 years in the use of PDAs for recording data, and we compare the benefits of recording field data electronically with those of data entry on paper sheets.

Regardless of the data collection medium, using standardized data forms has several advantages:

1. Fields for all data required for a given study are shown.
2. Omissions or errors during data collection are reduced.
3. Measurements to be used for a particular type of data (e.g., cm or mm) can be specified.
4. Others may be encouraged to use the same data fields when carrying out similar studies.
5. Standardization of data entry from form to computer increases.
6. A "permanent" record of the field event is created, regardless of whether the data are transferred into another digital format.

Our experiences with digital forms have shown that the benefits from their use, across the board, outweigh any problems they may create. Nevertheless, not having paper copies makes many researchers nervous. They always ask, "What if the PDA breaks, the batteries die, or it gets stolen?" All of these things could happen, and the investigator must take standard precautions to prevent them from occurring, including carrying pen and paper for backup in case of problems. Losing a PDA means losing all the contained data, but that would also happen if a field notebook were lost or stolen. In this section, we describe our experiences using PDAs to record data, as well as the instant backups for data now available. The greatest benefits of using PDAs are the reduction in numbers of data-entry errors made in the field, the decreased need for data entry when back from the field, and the significant control over standardization that is necessary for most field studies involving multiple data collectors.


Paper Data Form

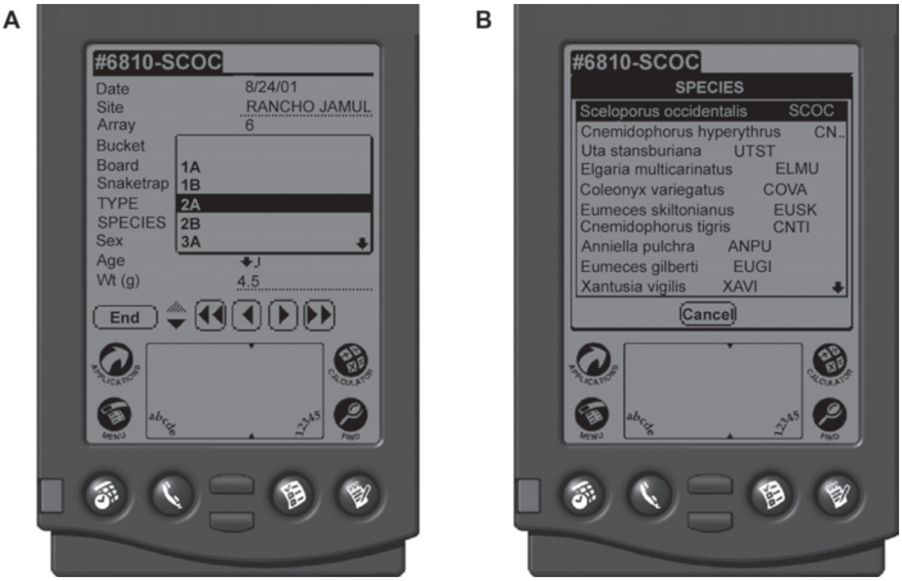
Date ________
Site Name ________
Array Number ________
Bucket Number ________
Snake Trap # ________
Species ________
Sex M/F Age A/J/?
Weight (gms.) ________
Length (mm/cm) ________
Marks ________
Toeclip number ________
Recap yes/no?
Collector ________
Disposition released/dead/escaped
Tissue sample yes/no?

Digital Data Form on Handheld Computer

#6810-SCOC
Date 8/24/01
Site RANCHO JAMUL
Array 6
Bucket
Board
Snaketrap
TYPE
SPECIES
Sex
Age J
Wt (g) 4.5

1A
1B
2A
2B
3A

FIGURE 19 Handheld platform for collecting data that displays a digital equivalent of each data field from the paper data form. In this display configuration, only 11 data fields can be viewed on a single screen; tapping the down arrow (▼) will bring the remaining fields into view.



#6810-SCOC
Date 8/24/01
Site RANCHO JAMUL
Array 6
Bucket
Board
Snaketrap
TYPE
SPECIES
Sex
Age J
Wt (g) 4.5

1A
1B
2A
2B
3A

#6810-SCOC
SPECIES

Sceloporus occidentalis	SCOC
Cnemidophorus hyperythrus	CN..
Uta stansburiana	UTST
Elgaria multicarinatus	ELMU
Coleonyx variegatus	COVA
Eumeces skiltonianus	EUSK
Cnemidophorus tigris	CNTI
Anniella pulchra	ANPU
Eumeces gilberti	EUGI
Xantusia vigilis	XAVI

FIGURE 20 Handheld platform displays. (A) A short pop-up list, which works well when the number of options is limited. (B) A look-up list, which may serve as a better drop-down list with fields that have more choices.

From Paper Forms to Digital Forms

After reviewing the various small, handheld computer options, we chose the Palm Operating System and an application called "Pendragon Forms" as the starting point for a pilot project to test the capability and accuracy of collecting field data with handheld computers. These items are available commercially (Appendix II) and designed to work together to build forms for data collection; they appeared to us to provide the most effective solution to our task. Data collection forms are developed on a desktop or laptop PC with the Pendragon Forms application and then distributed to the handheld units through the handheld's synchronization process. Data entered into the handheld can be downloaded into an MS Access or SQL (Server Query Language) table on the desktop PC during the next synchronization.

In the design mode, the developer specifies the names of the data fields, the types of data to be recorded in each, and the order in which the data fields appear. For basic features, no previous computer programming skills are required. Each data form produced has unique identifiers associated with the name of the form, which enable the program to route the data from the handheld back to the appropriate table on the desktop computer. With a little effort, a simple form that will run on the handheld computer can be created data field by data field (Fig. 19). Using the tools provided in the Pendragon Forms development window, features that facilitate data entry can be built into the form design. For example, drop-down lists can be used to standardize data and eliminate the need for retyping them (Fig. 20).

We deployed this system of data collection in the field in 2001 with immediate positive results, including minimizing the amount of traditional data entry required before the data could be used. Once the data were entered into the handheld, they were downloaded directly into an MS Access format where any of the tools available within MS Access can be applied. From this point, the data can be exported into MS Excel or ASCII format.

PENDRAGON FORMS

Pendragon Forms offers 23 data field types (Table 7), most of which are based on data-field types available in MS Access. Some of the Pendragon Forms field types are combinations or derivatives of multiple MS Access field types. Additional information about Pendragon Forms and more detailed descriptions of the individual field types can be found at http://www.pendragonsoftware.com/.

BENEFITS OF DIGITAL FORMS

One of the immediate benefits of using a handheld to record data is the reduction in time spent sitting at a computer transcribing data. The data are entered into the handheld in the field. With the handheld connected to the desktop or laptop PC, the push of a single button begins the data transfer process, with the data from an individual form being sent to its corresponding MS Access table.

As with paper forms, digital data forms can be tailored to the needs of an individual project. With some practice, users become familiar with the data entry process and the flow of the digital data form. Even sketches and diagrams that are made on paper can be collected electronically and transferred to the desktop PC.

With some basic and advanced programming, a certain degree of *error trapping* can be built into the data collection process at the point of collection, helping to improve the overall quality of the data set. For example, minimum and maximum value ranges can be specified for numeric fields. If a user enters a value outside of this range, he/she is notified while still in the field and in the best position to verify or correct it. Predefined options in a data field help to standardize responses, making it easier to analyze the data in that field. Translating someone else's handwriting is no longer an issue. Data fields that are most important to a study design can be set as required fields. If a user attempts to exit a record without entering a value into a required field, an error message prompts the user to supply the required information.

Data can be duplicated or backed up more quickly than ever before. With the mobile memory devices that are available for the various handheld units, the data can be backed up in the field or while moving between field sites. At that point, two copies of the data exist, one on the handheld and one on a memory device. Once the handheld is downloaded to the desktop or laptop PC, an additional copy of the data is produced and ready for use. With paper, a week could elapse before the data sheets can be copied and the data entered.

TABLE 7

Examples of Data Fields Available in the Pendragon Forms Application[a]

Freeform text
Numeric
Popup list
Lookup list
Date & time
Date only
Time only
Subform list
Currency
Yes or no checkbox
Option 1 of 5
Multi-selection list
Exclusive lookup list
Time checkbox
Section
Jump popup
Single subform
Signature
Button
Read-only text
Completion checkbox
Slider field

a. For additional information, see www.pendragonsoftware.com/.

Relational Digital Forms

The data are entered in the handheld as they would be entered into a flat-file spreadsheet application. The only difference is the place of entry, which can be either in the field or in the office. A single record is generated for each animal encounter. Everything about that capture is recorded in the same table. If an animal record needs to be analyzed with another data set, it must be reconfigured by hand.

Pendragon Forms can be taken a step further and formatted to run much like relational databases. Information about the study site can be stored in one table, data about the survey effort in a separate table, and animal data in a table with information only about the animal. The records from one table are linked to the appropriate records in a separate table by a linking or primary data field (Fig. 21). In this manner, animal records can be analyzed using any of the data fields collected to describe the study site without having to store the data with each individual animal record. We have developed forms that function more like relational databases for several projects,

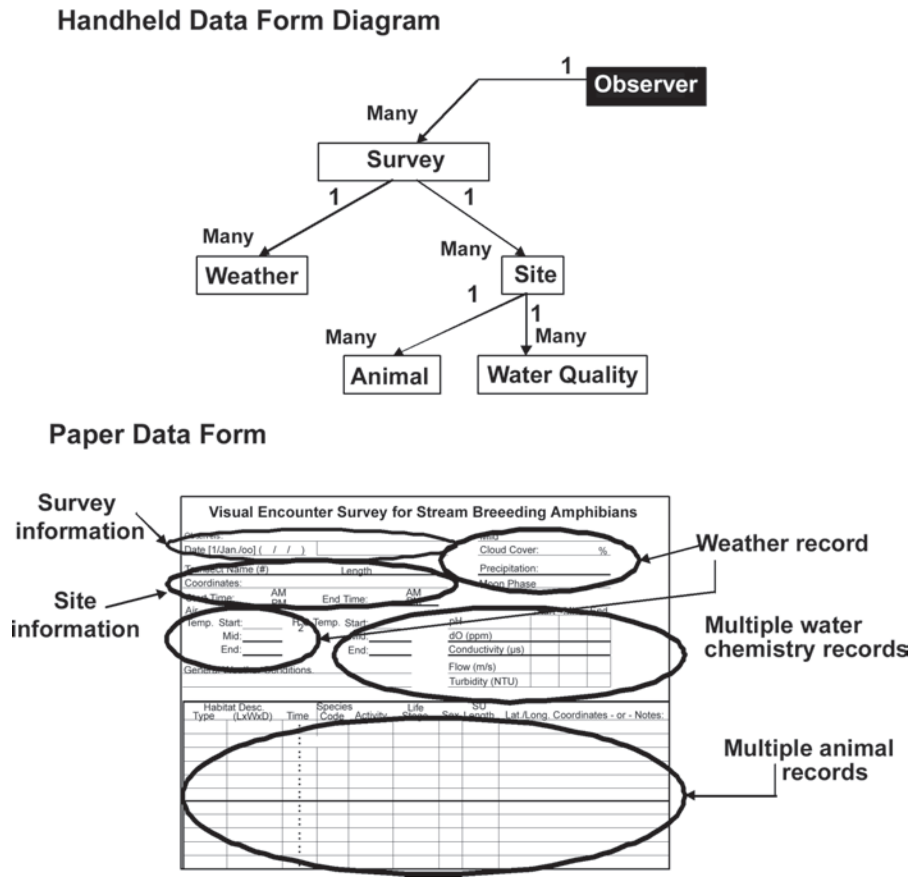


FIGURE 21 A relational database format can be built using Pendragon Forms and run on a handheld computer. Paper datasheet formats work well with a relational database model.

including pitfall trapping, stream surveys, turtle trapping, and desert tortoise transects, all with excellent results.

Managing Users, Forms, and Data

Pendragon Forms is designed to manage multiple users, including the selective distribution of forms and records among handheld units and the designation of handheld users who may add and delete data. Each handheld unit has a unique name. Using those names, a manager can determine which forms go to which handheld units. Field technicians doing one survey can have forms different from those of someone performing a separate, different-style survey.

A project manager can determine what a handheld user can enter on or delete from an individual form. For example, he/she may restrict a user from deleting or adding records or from modifying or overwriting existing records in the database computer. Handhelds can be set so that records generated by all users are shared by everyone by downloading them to all individual units. Alternatively, the program can be set to keep individual records only on the individual handheld.

Programming Options

BASIC

Many basic programming features are available in Pendragon Forms and are outlined in the user manuals. Two especially useful features are the cascading lookup and the automatic entry of the date and time.

A *cascading lookup* requires two data fields. The first is often a popup list that serves as a limiting selector. The second field is a lookup list. The choice that is selected in the pop-up list determines the selections that appear in the lookup list. An example of a cascading lookup would be the selection of a species name. In the popup-list field, the user chooses the type of animal species that he or she needs to find (e.g., lizard, snake, crocodilian, or turtle). When the user enters the second field, or lookup list, only animals of the selected type are displayed. This enables the user to select from a list of 10 to 20 species, rather than a list of 100 species. These lists are predefined and can be limited to species one might likely encounter during a survey, again reducing the possibility of errors.

Three fields, “Date & Time,” “Date Only,” and “Time Only,” can be set so that they are filled automatically. The programmer determines whether this occurs as soon as a new record is created or each time that the record is opened. Values reflect the time and date set on the handheld; if the internal clock for the handheld is incorrect, entries based on it will also be in error.

ADVANCED

With some background in programming, an investigator can set Pendragon Forms to perform some advanced functions, including downloading GPS data, error trapping with QA/QC (quality assurance/quality control) procedures, and automatically looking up toe-clip numbers. Data strings from a GPS contain information about latitude and longitude, elevation, estimated position error (EPE), and the datum (i.e., the standard geodetic reference system used when latitude and longitude are determined, e.g., NAD27, NAD83, or WGS84) that the particular GPS uses. The data strings, which are based on National Marine Electronics Association (NMEA) standards, vary depending on the GPS model used; special programs may be needed to accommodate them. Most often, the desired data are buried within the data string among other characters. Advanced scripts can be written to process the data strings to render the desired data. Ideally, a single field is used to activate the GPS download, which then populates five other data fields: latitude, longitude, elevation, EPE, and datum. Currently, position data collected with this system are only available in latitude and longitude units. More information about GPS data string availability and translations can be found at http://home.mira.net/~gnb/gps/nmea.html and http://vancouver-webpages.com/ peter/ nmeafaq.txt.

Setup procedures for QA/QC error trapping and for automated retrieval of toe-clip data are more complicated, and we will not cover them here.

Materials, Hardware, and Software

Various handheld units, including those that run on the Palm Operating System or Pocket PC platform, can be used. The desktop PC needs to run Windows 2000 or newer, as well as the synchronizations software appropriate for the PDA (see Pendragon website [Appendix II] for additional information on hardware and other requirements). MS Access is not required but can prove useful. Other equipment used includes modems, backup memory devices, and waterproof cases (most handheld computers are not waterproof). Before returning from the field, memory backup is an absolute must!

Limitations

Handheld units are neither desktop nor laptop computers. They are simpler computers that are slower and have less memory capacity. Most handheld units run at about 1 percent of the speed of a modern desktop computer and have a minimum of 8 megabytes of memory. Nevertheless, these machines are fast enough to do what an investigator needs, and they have more than sufficient memory for regular data entry. The processor speeds of handheld units should improve as newer models and operating systems are introduced. As with paper data forms, the possibility of data entry errors exists, and users can unknowingly record incorrect information. They may enter the wrong species code, select an incorrect option, transpose a set of numbers, or mistype weights and lengths. These types of error may never be eliminated from the data collection process, but investigators can take steps to minimize them.

Some investigators eschew the use of handheld devices in the field, sometimes because they are reluctant (or even resistant) to using computers or find the idea ill conceived. The type of data to be recorded, as well as the data collection process, can also interfere with the collection of digital data. This format works best when the user controls the flow of data, that is, if the same person generates and records the data or if one observer calls out the data to one recorder. If multiple observers call data to a single recorder, she or he may not be able to navigate between the individual records quickly enough to keep up with the data flow. Likewise, if the data come in very quickly, the person recording them may be better served by a familiar data sheet.

Conclusions

Careful consideration and planning should go into the development of all data forms, whether in paper or digital format. The questions that will be asked of a data set should be defining factors in the design and implementation of a digital data form. In addition, data forms are likely to evolve and may need to be revised and updated after use in the field. Investigators should also consider the goals, resources, and limitations of their research carefully in order to select the hardware and software applications that best meet their needs. The choices presented here are by no means the only options available but simply represent what was best for our work. Finally, a disclaimer: handheld computers are not the solution for every person or situation. They will not solve every problem in an investigator's life or research. They do, however, do a great job of reducing the amount of data transcription required.

Databases, Metadata, and Integrated Data Management

Christopher Brown and Robert N. Fisher

One end goal of a study is to analyze and synthesize the data collected in an effort to understand their meaning. Most data from field studies are analyzed in an electronic format, using modern software packages for models and statistics. Consequently, how the data are stored is important and should be considered simultaneously with protocol development. Data definitions and data storage should reflect how the data are collected in the field and how they ultimately will be analyzed. By thinking backward from final analysis to data collection, an investigator can strengthen the latter effort by clearly identifying the data that must be collected (*parameters*), clarifying the definitions of the data fields, and setting appropriate values or boundaries for data collected in those fields (*domain*), regardless of the storage medium. Consider, for example, a study that requires length measurements (parameter) for each animal observed. The definition of the parameter might be "tip-of-the-snout-to-vent length in millimeters," and the domain for a given species might have bounds such as "integers greater than 30 and less than 120."

Data storage can take many forms, from notes in field notebooks to an industrially served relational database. Each method has associated costs and benefits. For example, the startup costs for recording and storing data in field notebooks are less than those for recording and storing the data electronically (paper notebook vs. computer and software). However, the cost in time and (potentially) personnel that will be required to record, mine, and proof data from field notebooks and transcribe them into a well-developed relational database (see next section) for analysis will soon surpass any initial savings.

Relational Databases

A *relational database* (see Box 2) is one in which data are stored in multiple subject-specific tables rather than in a single large

BOX 2 RELATIONAL DATABASES

Databases are files that store field-collected (or other) data such as observations, dates and times, species observed, specimen variables (e.g., weight and lengths), weather and habitat variables, and so forth. Databases can be anything from field notebooks or spreadsheets (Microsoft Excel or text files) to database software files (e.g., MySQL or Microsoft's Access and SQL Server). Setting up database software, which comprises and is referred to as a *relational database management system* (RDBMS), often requires substantial effort; however, a properly configured system can provide superior data consistency and result in tremendous cost savings in the long term. *Normalization* is the process of creating tables to store specific types of data and relating them to other tables. For example, a study may have included many surveys, and each survey may have produced many species observations. These three subjects (studies, surveys, species) can be stored in different tables with defined relationships. To record data on a species observation, for example, an investigator first needs to record a survey event with which the species data can be associated; likewise, before the survey event can be recorded, it will have to be associated with a study. Balancing this normalization process with practical data collection is critical to a well-developed relational database.

table. The tables are related to each other using common fields that identify specific records. Relational databases have several advantages for data storage, including size, speed, and efficiency. Data that are stored in a well-developed database are also immediately accessible. They can be queried repeatedly for multiple values across many fields, reduced, and/or reassembled in many different ways quickly and easily, without having to physically examine the entire data set (either on paper or in a spreadsheet) to look for representative values and types of data. Many database applications, ranging from shareware to industrial application software, provide query tools. Relational databases are a norm for data storage, for which extensive technical support is available, and they are recognized as standard by data administration professionals, who will be able to interact with the data easily.

The inherent level of error checking in relational databases is much greater than that of spreadsheets or paper data. By separating the spatial and temporal information and creating relationships between the categories of field data, collection errors can often be avoided. For example, recording an individual surveyor in multiple locations at the same time or twice entering the data from a single survey at a particular location and time can be detected easily or even precluded. Relationships also clarify the associations among kinds of data. Particular data may pertain to a specific location at a specific time, or they may have a larger spatial and/or temporal extent pertaining to multiple sites and/or surveys, which may be obscured when the data are in spreadsheet format. For example, data that are site covariates should not be stored as survey covariates, and vice versa, yet these data are often stored for each record in a spreadsheet.

Storage in a relational database conserves space while allowing for redundancy and enhanced security. Electronic data can easily be sent to multiple locations across thousands of kilometers in a few seconds. An equivalent degree of data backup with paper copies would be time and cost prohibitive.

Data persistence is another great benefit of well-developed relational databases. A robust relational database with well-defined fields, relationships, and metadata allow the data to be useful long after the study for which they were originally collected has been completed. Relational databases (especially those utilizing Extensible Markup Language [XML], Structured Query Language [SQL], or Open Database Connectivity [ODBC] connectivity) can easily be maintained in current software and can often be migrated forward even after many years of neglect. This attribute, combined with the ability to quickly create backups, print hardcopy sheets, and export data in multiple formats that can be read by multiple applications, also increases data persistence.

Metadata

The terms *Geospatial information* and *metadata* refer to information that describes data sets, including information about the geographic extent (e.g., Geographic Information System coordinates) of a study or survey, as well as records of the collector(s) involved, when and why the data were taken, methodologies used, and a summary of the data in the database, which may include multiple datasets. Such information can be stored more effectively within a database than elsewhere (see Box 3). Tables can be created individually to hold this information, or geospatial software packages can actually create and manage these types of data within a personal geodatabase. Storing this information within the database that includes the dataset it describes provides for quick spatial visualization, summarization, and sharing of data with less concern for misrepresentation or loss—all necessary data definitions, protocols, and spatial attributes are stored in the same file. Once this structure is implemented, tools for data reduction, visualization, and analysis can easily be created and supported.

BOX 3 METADATA

Metadata are data about data. These data describe a study and include such things as the period during which a study took place, names and contact information for owners of lands where a study was done as well as for collaborators, detailed descriptions of protocols used for every activity, and definitions of each type of field data, including values that may be recorded. Many agencies maintain catalogues of metadata so that they can easily reference studies conducted on their properties or with their funding. Other organizations catalogue metadata for studies that fall into categories of interest to them. The categories can be as specific as impacts of roads on reptiles or as general as the ecology of particular species. Generally, the information in the catalogues is not integrated, and catalogues do not necessarily store or present data; they do provide a source that others can use to locate studies (and, presumably, data sets) that have been carried out on specific topics.

Successful integration of data can be accomplished when these elements are all brought together (see Box 4). A user accessing a single relational database that stores the metadata, separates the spatial and temporal information, and relates the data using definitions and collection methods can retrieve meaningful ecological data across multiple projects regardless of geographic or chronologic extent.

With the pace at which analytical software and data storage change, it is important that data be stored in a format from which they can be accessed and used both now and in the future. By integrating data from multiple studies in a relational database, the data can be much more meaningful. For example, data from reptile visual encounter surveys and from pitfall traps may be more informative when combined, and even more so when integrated with habitat data from other studies. In this way, the lifetime of the data will exceed that of the study for which they were originally collected.

Data Quality Assurance and Quality Control

Andrea Atkinson, Carlton J. Rochester, and Robert N. Fisher

Developing a Quality Assurance Plan

Maintaining a high level of quality during data collection, management, and interpretation can be challenging, but it is essential for the success of any program. Investigators wishing to ensure that data collection is repeatable, comparable through time, and suitable for analysis should invest in the development of a quality assurance plan. This can be relatively simple for small, short-term projects, but it will likely become increasingly complex for more complicated, long-term programs. The first step in developing a quality assurance plan is to determine its goals and anticipate potential problems. Some basic questions to consider include the following:

1. What is the desired level of data quality?
2. What known problems exist in the data collection and recording phase, what steps are needed to ensure the proper calibration of equipment, and what impact may personnel have on the process?
3. How can these problems be minimized before, during, and after data collection?
4. What steps can be taken to promote a consistent data collection effort over time and space and among multiple observers?
5. What techniques or procedures will be employed to review the data, and what actions can be taken to reduce future problems?

Some key practices that help to ensure high-quality data are (1) the early establishment of clear communication among the head of the project and those collecting data in the field or laboratory, (2) the implementation, documentation, and maintenance of a chain of supervision and responsibility, (3) comprehensive testing of each protocol prior to its adoption, (4) thorough training for project personnel, and (5) a systematic review of the procedures and output of the study and data.

Elements of Quality Assurance and Quality Control (QA/QC)

A number of steps can be taken to maintain adequate data quality. Some are general and apply to most studies. Others may be specific to a particular study. Below we list some of the former:

1. Development of a study plan with clearly defined objectives that has been reviewed by a biometrician.
2. Development and/or use of protocols that allow easy standardization across observers and time (i.e., protocols that minimize observer bias or provide an estimate of observer bias, such as double-observer methods).

BOX 4 INTEGRATED DATA MANAGEMENT

Integrated data management is the storage and maintenance of data from different sources or studies in a format that allows them to be queried and reduced together. Setting up the database requires considerable forethought, especially during the normalization process. One study, for example, may have involved repeated visits to a single site, whereas another study may have involved single visits to many locations. Nevertheless, the data from the two studies can be integrated if the site and survey information are structurally separate and the observation data are related to the two independently. It is also important to record negative data (e.g., when no organisms [= reptiles] are detected) in this type of design to provide an accurate measure of survey effort and for use in presence-absence species models.

3. Designation of standard measurement units and nomenclature, followed by the distribution of these instructions to all involved to ensure that the meaning and use of terms are the same for everyone. The location “Cabrillo National Monument at Point Loma,” for example, could be recorded as “Point Loma,” “Loma,” “Pt. Loma,” or “Cabrillo National Monument” by different observers if a standardized name is not selected. A well-designed database should include tools that encourage the standardization of such data fields, greatly reducing this problem.
4. Testing of field protocols, data recording methods, forms, and record-keeping techniques before expanding their use to a large project.
5. Distribution of the objectives of the monitoring program, the protocols to be used (including how to handle various problems that may arise), the units to be used for each measurement, the procedures for recording data, QA/QC procedures, and answers to commonly asked questions to everyone involved in the project before it is initiated. Field personnel should understand the “why” of a protocol as well as the “how” and “what” of the tasks they need to accomplish.
6. Holding regular training sessions to standardize methods across observers and time.
7. Development of regular schedules to check the calibration of field equipment such as water-quality loggers, temperature loggers, and a mass scale.
8. Utilization of data collection techniques that minimize the frequency of errors and forgotten fields.
9. Having field observers double-check their own data, preferably when they are collected.
10. Double-checking (proofing) all data that are recorded on paper and later transcribed into a computer file.
11. Having a supervisor, data manager, or project leader check the data regularly for obvious errors. In some cases, the data management system can be designed to flag data outliers (such as measurements recorded in centimeters rather than millimeters) for review. Supervisors should review data collected by new observers on a daily basis initially and less frequently, but regularly, after that. Data review should not be left until the end of the field season, by which time memory of the events that the data are meant to represent may have faded.
12. Maintenance of information on the origin and status of the data, such as who collected them, who entered the data into the database and when, and documenting the current level of review; such information serves as an important “chain of custody” for the data.
13. Synchronization of data maintained in multiple places, such as in a local database and a regional database. All versions of the data must be kept current, with changes made in one data base quickly transferred to all other databases.
14. Development and maintenance of appropriate metadata along with the database.
15. Establishment of security measures on the database to ensure that only authorized personnel have access to and the ability to modify particular data.
16. Tracking the level of QA/QC associated with a given set of data and ensuring that end users are aware of its QA/QC status.
17. Maintenance of an open connection between the individuals who analyze and interpret the data and those who collect the data in the field.
18. Development of a quality assurance plan or standard operating procedures manual.

Quality Assurance/Quality Control for a Pitfall-Trap Study

To illustrate a few of the steps that might be involved in a quality control plan, we present an example of the data review that would be done for the pitfall-trap study design discussed in Chapter 13, “Standard Techniques for Inventory and Monitoring” (see “Pitfall-Trap Surveys”). High standards of quality assurance and control of field data require actions at multiple stages of the data-collection process. An essential piece of quality assurance consists of a same-day review of the data records, whether collected on paper sheets, handheld computer, or by some other electronic means. Additionally, if tissue samples are collected, the vial labels and contents should be checked for accuracy against the animal records. The field technician who collected the data should be the one who checks for obvious errors or omissions. This should be done immediately after returning from the field while the captures and events of the day are still fresh in the technician's mind.

One of the next steps in proofing pitfall-trap data involves checking the accuracy of the toe-clip numbers used to mark the individual animals that were captured. Species records and toe-clip charts are compared to ensure that all numbers that were used in the field have been marked as such and removed from the list of available numbers. Next, toe-clip numbers are checked for replication. If the same toe-clip number was mistakenly used twice for the same species, this is documented in the notes field of each species record. Finally, the data are checked to make sure that the correct toe-clip numbers were used with the correct species. Occasionally, mistakes are made and the toe-clip numbers for one species are used out of sequence for an alternate species. Any abnormalities are checked with the field technician, noted, and/or corrected.

For our studies that use pitfall-trap data, we have automated many QA/QC tools, building them into the database. For example, one part of the review is to proofread the animal data. This includes reviewing species identifications and measurements. Tools located within our database flag records of weight and length measurements that are not appropriate for the indicated species. The listing of a previously unreported species at a study site also produces a warning flag, prompting a review of the record with the field technician. The development of such QA/QC tools requires an in-depth familiarity with the data, survey protocols, and study design, combined with an understanding of what constitutes an error or deviation from the desired results.

CHAPTER FIVE

Finding and Capturing Reptiles

LEE A. FITZGERALD

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Introduction

Successfully finding and capturing the reptile species of interest is clearly a fundamental requirement for a project's success. It is also one of the most enjoyable and rewarding aspects of the research of a field herpetologist. Scientific questions about the occurrence, abundance, and ecology of a species cannot be answered, however, if a species cannot be reliably detected by some reasonable method. In this chapter we review methods that herpetologists have found to be particularly useful and effective for sampling reptile populations. After general considerations, we discuss the methods, which are organized into two groups. One group includes methods that are broadly applicable to many taxa, for example, noosing and pitfall trapping. The second group includes techniques used to sample particular major taxa.

Obviously, standard methods do not exist for detecting each of the more than 9,100 reptile species (Uetz et al. 2011; see Chapter 2, "Reptile Diversity and Natural History: An Overview"), and biologists are likely to face the problem of monitoring species that have never been studied. Nevertheless, the information we present should serve as a guide for adapting widely applicable techniques to poorly known species and novel situations. The diversity of body sizes, habitats occupied, foraging modes, and activity patterns among the Reptilia (turtles, crocodilians, squamates) means that generalized capture methods are unlikely to work for all species. Many lizard and snake species are easy to catch in pitfall and funnel traps, for example, whereas others are not. Even within single families, species vary in their detectability and their vulnerability to capture. Consequently, herpetologists planning to sample a particular species must be mindful that techniques that work for one group of species may not work as well for another. Resourcefulness, combined with field experience, goes a long way toward identifying the best capture technique(s) for each situation. A variety of specialized techniques invented to capture reptiles in special situations are covered in Chapter 11, "Techniques for Reptiles in Difficult-to-Sample Habitats").

General Considerations

Preliminary Checklists and Species Assessments

During the planning stages of a project, investigators should consult (or compile, if necessary) a list of species expected to occur in the study area, to help identify appropriate capture techniques. Reports in the literature and records from natural history museum collections (see Appendix I for a list of institutions with significant collections of reptile specimens) can be used to create a preliminary list of likely species. A review of the literature on the reptile species one expects to encounter can also provide information on observation and capture methods that have worked for those species in other studies.

Scope of the Study

A critical consideration when planning capture methods for a particular project is whether all or only a few reptile species are to be inventoried or studied in depth. Although additional information on any reptile fauna is always desirable, accomplishing complete species inventories in a timely fashion requires a full-scale application of every class of technique and

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is, consequently, time intensive, labor intensive, and expensive. In contrast, projects targeting specific species or components of a reptile fauna need only employ methods relevant for those taxa. Because funding, time, and personnel are limiting factors for essentially all projects, devoting resources to additional sampling techniques to carry out a species inventory can be a drain on a project primarily focused on the study of select species. Moreover, some methods employed in all-species inventories can interfere with long-term monitoring objectives for specific species. For example, inventory methods that disrupt microhabitats and remove individuals from a population may introduce unquantifiable biases into population-monitoring studies. Nevertheless, nearly every trapping method results in the incidental capture and observation of nonfocal species, which contributes to the growth of species lists for an area. A good example is inventory information gathered during long-term ecological research on targeted species at the Savanna River Ecology Laboratory sites in South Carolina. Although the studies were designed for specific taxon groups, incidental information gathered during the course of the studies allowed for the monitoring of reptile assemblages in the area. The pros and cons of different sampling designs are discussed in detail elsewhere (see Chapter 3, “Study Design and Sampling”).

When a study includes both a general inventory of species and the monitoring of targeted species, the inventory should be carried out first. Opportunistic collecting of species for the inventory can be continued during the subsequent targeted monitoring. Another obvious planning consideration is whether or not animals will be removed from the study area and preserved as scientific specimens or if they will all be released after examination. Similarly, methods that are likely to injure a study organism can result in observer-induced mortality and introduce bias in capture-release studies. Dislodging lizards from a perch with rubber bands is a good capture technique (see “Projectiles: Rubber Bands . . . ,” below), for example, but it can kill small animals, so the method is too risky to use if lizards are to be released unharmed. The converse is also true—if the goal is to collect voucher material, then using labor-intensive live-capture techniques is unnecessary. Hunting with approved weapons (e.g., firearms, compressed-air rifles, sling shots, blowguns) is probably a more efficient method of capture, and it avoids all issues associated with caring for live animals that are destined from the outset to become specimens.

Life History and Ecological Correlates to Capture Methods

A recurring theme throughout this volume is that knowledge of a target species’ natural history enables an investigator to predict where the species may occur and, one hopes, how it can be observed or captured. The landscape of a study area and the habitat affinities and activity patterns of target species influence where, when, and how reptiles can be observed and captured. Determining how to observe and capture arboreal species when inventorying an area of tall forest, for example, requires considerable forethought. Inventorying such areas will also require use of methods appropriate for terrestrial and fossorial species. Similarly, employing techniques and protocols that enhance detection of nocturnal species is important. In some situations, species may be readily detectable if they use predictable retreat sites, such as branch tips used by diurnal *Anolis* lizards for sleeping at night, crevices used by Common Chuckwallas (*Sauromalus ater*), and burrows occupied by tortoises.

Detecting, Counting, and Sampling Squamates

Searching on Foot

Most squamates are found simply by walking through suitable habitat and actively searching for individuals that are active or in their refugia. On a typical outing, the accomplished herpetological field collector is equipped with a few plastic bags for small reptiles, one or several cloth bags for larger snakes and lizards, a notebook and pen, a camera, and often a tool for turning rocks and logs. A potato rake is an efficient tool for searching through leaf litter and for turning and breaking apart rotting logs. Durtsche (1996) developed a novel double-ended tool; he attached a telescoping car antenna that had a sticky pad mounted on its distal end to the handle of a potato rake. He used the potato rake to turn objects, and when he spotted a small lizard, he quickly captured it with the sticky pad. This technique facilitated the successful capture of small lizards that were otherwise difficult to sample (Durtsche 1996). Several makes and models of sturdy snake hooks can also be used to turn rocks and logs (see Appendix II).

In the Peterson Field Guide, *Amphibians and Reptiles of Eastern/Central North America,* Conant and Collins (1998) provided a classic overview of techniques for catching and handling these animals, with illustrations of how to hold live reptiles (see “Handling Live Reptiles,” in Chapter 8) and the common tools used in this endeavor. Catching reptiles by hand takes a lot of practice. Most investigators grab reptiles by hand; they should be aware that reptiles may bite. Some snakes, although nonvenomous, can bite hard enough to draw blood.

The number of reptiles encountered by an investigator on foot is a function of the time spent in the field and the intensity of the search. The best collectors tend to stay out the longest, actively walk long distances, and have a good feel for the kinds of microhabitats most likely to be productive. While searching for reptiles on foot, a collector must watch for active lizards and snakes as well as look under suitable cover where reptiles may hide. Many species take refuge under rocks or fallen logs, inside rotting logs, and under the bark of standing snags. The hook or rake is useful for turning rocks and tearing apart rotten logs. Microhabitats are disturbed when searching for reptiles, and it is important to return logs and rocks to their original positions. Piles of boards, sheet metal, and other debris, as well as collapsed buildings, provide excellent hiding places for reptiles because refugia are abundant. Such habitats can easily be taken apart and searched.

Park rangers and I (LAF, unpubl. data) captured ctenosuars and Green Iguanas (*Iguana iguana*) at Laguna El Jocotal National Park in El Salvador by hand and with nooses (see “Noosing,” below). One or two individuals with noose poles climbed a tree, noosing lizards whenever possible. The iguanas and ctenosaurs retreated to the ends of branches and finally jumped to escape. Investigators on the ground caught them in mid-air or chased and caught them on the ground. This technique is feasible only if several enthusiastic catchers are available to surround the tree. Also, if a tree is over water, the lizards may dive in and escape. We caught hundreds of individuals of each species this way over a 1-year period.

Noosing

A noose is a pole with a line at one end; the free end of the line is tied back onto itself with a slip knot, to form a noose. The noose is slipped over the head of the target animal and quickly tightened to effect capture. Many herpetologists carry a noose pole for catching active lizards. Noosing is not efficient for most nonvenomous snakes, which are easier to catch by hand. Safe capture and handling of venomous snakes require special techniques (see "Handling Live Reptiles," in Chapter 8).

Noose poles can be made from fiberglass, graphite, or cane fishing poles. Nooses are made from dental floss, fly-line backing, silk suture thread, cotton thread, or other suitable material. We recommend against using monofilament line because it tends to constrict tightly and may cut a lizard's skin. Collectors tend to develop personal preferences for the length and stiffness of the noose pole, the material used for nooses, and even the type of slip knot used to create the noose!

The principle of noosing is simple, but success requires practice. The noose should hang open, suspended a few centimeters from the end of the noose pole. The investigator slowly moves the pole into position over the lizard, slips the noose around its head, and then snaps the pole upward, tightening the noose and lifting the lizard into the air. She or he then grasps the lizard gently with the free hand and loosens and removes the noose while holding the pole under the arm or after laying it on the ground.

Noosing is a scalable technique that can be adapted to reptiles of various sizes. Nooses made of thread, fly-line backing, or unwaxed dental floss and tied with a bowline knot work well for most lizards weighing 75 g or less. Tiny hatchling lizards can be captured with nooses made of fine sewing thread and attached to a lightweight pole. Nooses of rope or nylon cord attached to long poles have been used successfully to capture crocodilians and large lizards, especially iguanines. Commercially available locking snares and cable snares attached to sturdy noose poles are also used for capturing crocodilians (see "Capture Equipment" in Appendix II).

Strong et al. (1993) modified the noosing technique by tying a meal worm to a monofilament loop. When lizards bit the mealworm, the loop was flipped into the air. The lizard usually remained clamped to the mealworm and could be grabbed; occasionally it fell (or jumped) to the ground and had to be caught by hand. The investigators had a 93 percent capture efficiency with *Cnemidophorus hyperythrus* [= *Aspidoscelis hyperythra*] (Squamata: Teiidae) and noted that the technique worked well with another teiid species and two phrynosomatids.

Projectiles

RUBBER BANDS

Large rubber bands, ca.12 × 1.5 cm (4.75 × 0.6 in), can be used as projectiles to stun lizards or dislodge them from perches so that they can be grabbed. The small, thin rubber bands typically used in offices are unsuitable. The technique is useful with lizards perched several meters up in trees, on rock walls or buildings, and in dense thickets. The rubber-band technique, in contrast to other projectile methods, is easy to learn and use. Looping two large rubber bands together so that they form a knot in the middle creates a longer and more efficient tool. One end of the rubber band is hooked on the thumb or index finger; the band is stretched with the other hand, aimed, and released to fire. It dislodges the lizard from its perch, and the investigator grabs it. Because the impact of the band or the substrate sometimes stuns, injures, or kills an animal, we advise against using rubber bands if captured lizards are to be released.

SLING SHOTS AND BLOWGUNS

Sling shots and blowguns are efficient tools for collecting some reptiles, although success requires a lot of practice. These implements can be used for animals larger than those collected with rubber bands, and they have a much longer range. Collectors develop preferences among projectiles of different sizes and hardness for use with different animals. Blowguns provide a handy method of collecting amphibians and reptiles, particularly lizards. They are most effective at distances of 5 to 10 m. As with a rifle, the longer the barrel, the more accurate the blowgun. A number of lightweight and accurate blowguns, projectiles, and accessories are available from various sporting goods and hunting supply stores for less than US$25.00. Commercially made stun darts are available from blowgun suppliers in packs of 25 for less than US$3.00 (e.g., Cabela's, Target Zone Sports; see Appendix II), and we recommend their use. However, blowguns that are as good as those available commercially (F. W. King, pers. observ.) can be constructed with inexpensive materials easily obtained from a local hardware store. A 305-cm (10-ft) section of 1.27-cm- (½ in-) diameter electrical conduit can be purchased for less than US$3.00 and cut to produce two blowguns 152.4 cm (5 ft) in length. A mouthpiece made by placing any rubber cup (used on the tip of canes or furniture legs) snugly over one end of the blowgun will prevent cutting or bruising of the lips. The cup is placed firmly on the end of the conduit and hammered with a heavy piece of lumber (a steel hammer could bend the edge of the conduit) to drive the end of the conduit through the rubber tip. The tip is then removed, trimmed, smoothed, and replaced.

Blowgun darts are of two types, blunt projectiles that stun or kill with the force of impact, and sharp darts or arrows that impale the target. Most biological specimens are collected using blunt projectiles. No. 4 cork-bark corks are a tiny bit too large to fit easily inside a ½-inch-diameter electrical conduit. However, if a cork is rolled or pinched before insertion into the blowgun, it slides easily but is snug enough to prevent air from escaping around its periphery. Cork size can also be adjusted as needed with sandpaper. Stoppers made from neoprene or rubber are not suitable, because their diameters are not easily decreased. However, cork projectiles do not have sufficient mass to stun a target animal, so collectors often drive a galvanized roofing nail into the small end of the cork until its head is flush with the cork surface. The sharp end of the nail does not protrude from the cork. The flat steel face and added weight of the nail are sufficient to crush the head of and instantly kill a lizard 20- to 40-cm long. Weighted corks are also ideal for stunning or killing snakes that have taken refuge in dense vegetation. Coachwhip Snakes (*Coluber flagellum*) up to 183 cm long have been stunned and caught this way. Corks can be painted fluorescent orange or pink to facilitate retrieval from leaf litter, vegetation, or rocks. Blunt projectiles can also be made from other materials. Plasticine, a nonhardening clay, is available in a variety of colors from any toy store. A wad rolled into a ball that fits inside a blowgun has sufficient weight to stun or kill the target reptile. Green grapes, which have sufficient weight to stun an animal without damaging it, also make effective projectiles in blowguns (J. R. Dixon, pers. comm.).

Sharp darts can be made by driving a large sewing needle or a heavy wire (e.g., from a coat hanger) with one end sharpened to a fine point through a no. 4 cork so that it protrudes 15 to 20 cm from the small end. With a strong puff on the blowgun, the tip of the dart can pierce 0.6-cm-thick (¼-in) plywood. Few field biologists use sharp darts because the impaled reptiles rarely die instantly and because they can get stuck up in trees. A cork (or other projectile) stuck in a blowgun is easily dislodged by dropping a pebble, bolt, or heavy nail into the blowgun and jiggling it up and down.

FIREARMS

Firearms have been used extensively to collect reptiles that are to be preserved as specimens. Using shotguns, rifles, and handguns is appropriate in some situations if approved by the administrative entities or institutions overseeing the research. Large species can be collected by traditional firearm hunting. A .22-caliber revolver loaded with #12 lead shot shells is an efficient tool for collecting lizards and snakes. The #12 shot will kill even very large snakes at distances of about 6 m. Shooting small lizards and snakes with a revolver at distances of 3 to 6 m causes surprisingly little external damage; small specimens shot at close range are visibly damaged. A .22-caiber-shot shell has a much longer range when fired from a rifle, but it also causes more damage to a specimen at close range.

Compressed-air rifles fire a single lead projectile and for all practical purposes are the same as small-caliber firearms. BB guns also operate on compressed air and fire a single small brass ball. BB guns can be surprisingly accurate and have been used to collect lizards at a range of 12 m (L. J. Vitt, pers. comm.). Many models of compressed-air rifles and BB guns are available at a wide range of prices (from less than US$30 to more than US$300) from hunting stores and toy stores.

Trapping

A variety of simple methods have been devised for trapping reptiles that are otherwise hard to observe or for use in inventories or studies with specific sampling designs. Below we review some common trapping techniques with wide applicability. The use of several of these methods as well as some specialized trapping techniques is discussed in greater detail in Chapter 13, "Standard Techniques for Inventory and Monitoring." A species' natural history can suggest which microhabitats it may use, but it takes ingenuity to lure animals from their refugia. Herpetologists routinely adapt and modify techniques to fit a particular situation. As with all field methods, resourcefulness is the key to success!

COVERBOARDS

Coverboards are simply sheets of plywood or other material that are laid on the substrate in suitable habitat to create artificial refugia that are attractive to many kinds of reptiles (see "Sampling with Artificial Cover," in Chapter 13). Reptiles that shelter under coverboards are not trapped but can move in an out freely. Consequently, they need not be checked daily, and maintenance required is minimal, especially compared to pitfall traps (see "Pitfall-Trap Surveys," in Chapter 13). Coverboards and pipe traps (see "Tubes," below) can be configured in grids for standardized sampling.

TUBES

Strong et al. (1993) successfully trapped open-habitat lizards in lengths of cardboard, plastic, or polyvinyl chloride (PVC) pipe that had one end closed with tape. They placed the tubes horizontally on the ground near bushes and then chased the lizards, which ran from their refugia into the pipes, where they were trapped. (Lengths of PVC pipe driven into the ground in wetlands retain water and provide good refugia for frogs, especially treefrogs. These standing PVC pipes rarely trap reptiles in wetlands; we mention them because many biologists sample amphibians and reptiles at the same time.)

SEINES AND DIPNETS

Seining and dipnetting are excellent methods for sampling tadpoles, salamander larvae, sirens, and amphiumas. Seines can also be used to remove turtles from shallow waters. A 1.8-m-long (6-ft), ca. 0.64- or 0.32-cm (¼–⅛-in) mesh seine and a dipnet should be standard gear for any herp collecting trip. Small sturdy seines that work well in water with dense vegetation can be fashioned from either metal or plastic mosquito screen.

ADHESIVE TRAPS

Glue traps for mice have been used in a variety of ways to catch lizards and snakes, and their efficiency in several habitat types has been evaluated in several publications (Bauer and Sadlier 1992; Rodda et al. 1993; Whiting 1998; Glor et al. 2001; Ribeiro-Júnior et al. 2006). The technique is simple; glue traps are placed on the desired substrate, and wandering reptiles get stuck in the trap adhesive. Whiting (1998) baited his traps with figs to increase capture rates of *Platysaurus capensis* (Cape Flat) lizards. Large species can be gently worked from the traps, but small lizards and species with fragile skin (e.g., geckos) usually require an application of vegetable oil (a small paint brush makes a good applicator) before the skin can be carefully loosened from the glue wherever the skin is stuck.

Some advantages of adhesive trapping include the ease with which traps can be arrayed on transects or in sampling grids, their suitability for placement in most microhabitats (including in trees), low cost, and easy transport. The primary disadvantage of adhesive trapping is the risk that the trapped animal will be injured or die. Animals die from stress, injury during removal from the traps, predation by ants and vertebrates while immobilized in the trap, and dehydration and overheating from exposure to the sun. Traps should be checked frequently to minimize mortality, which averaged 10.9 percent in one study (Glor et al. 2001).

Funnel Traps, Pitfall Traps, and Drift Fences

Lee A. Fitzgerald and J. H. Yantis

A funnel trap is a cylinder with a funnel-shaped throat at one or both ends (see "Pitfall-Trap Surveys," in Chapter 13). It is excellent for trapping reptiles in aquatic (turtles, aquatic snakes), terrestrial, and arboreal habitats, in part because only about 20 percent of the reptiles (and other organisms) that enter the trap find their way out. Many variations on the standard funnel-trap design, however, are even more effective. Yantis and colleagues (Farallo et al. 2010) designed a one-way double-funnel trap that captured significantly more reptiles and more total fauna. Less efficient single-funnel traps are sold commercially as minnow traps at sporting goods stores and bait shops. In aquatic settings, funnel traps are secured to an anchor (e.g., log, rock) on the shore or in the water; part of the trap must remain above water so that captured animals can breathe. Plastic bottles can be used as floats, but the investigator must ensure that the load of trapped animals does not exceed the buoyancy of the trap (J. R. Dixon, pers. comm.). Casazza et al. (2000) attached polystyrene foam floats to commercially available "eel pots" (double-ended funnel traps manufactured from hardware cloth especially for trapping eels) for surface collection of aquatic herps. Watersnakes enter traps on their own, especially after minnows have become trapped. The funnel entrance holes need to be just large enough to allow entry of large snakes and should not contact the sides of the trap. Appropriate bait can be placed in a funnel trap to attract specific target species.

Pitfall traps consist of an open can or bucket buried flush with the surface of the ground (see "Pitfall-Trap Surveys," in Chapter 13). Animals simply fall into them and are unable escape. Twenty-liter (5-gal) buckets make excellent pitfall traps; their nesting within each other also facilitates transport. Two #10 cans stacked one on top the other, with the bottom of the upper can removed, also work well. Holes must be drilled in the bottoms of pitfall containers to allow rainwater to drain. Pitfall traps should be covered with boards larger than the pitfall and elevated a few centimeters above it (Ferguson et al. 2008). Covers shade trapped animals, lessen the rain entering the container, reduce mesocarnivore predation, and provide enticing entrances for reptiles. Squares of 40.5 × 40.5 cm (16 × 16 in) cut from standard sheets of plywood cover 5-gal-bucket traps.

Adjacent pitfall traps are commonly connected by drift fences, which intercept wandering animals and prevent them from crossing the area between the traps (see discussion and figures in "Pitfall-Trap Surveys," in Chapter 13). Blocked animals often follow the fence and encounter a pitfall or funnel trap. Drift fences are usually buried in the ground to a depth of 15 cm, with an additional 40 to 60 cm above the ground.

Drift fences can be made of aluminum or galvanized flashing, vinyl flashing, hardware cloth, mosquito screen, polystyrene plastic sheeting, or silt screen (Malone and Laurencio 2004). The recently available vinyl flashing is as durable as aluminum flashing but weighs less. It is available where aluminum flashing is sold and is about the same price. Polystyrene plastic sheeting is more rigid than polyurethane and is available in hardware stores throughout the Neotropics. It is lightweight, affordable, and fences of any length can be made by joining the overlapping ends of sheets with lacquer thinner solvent, which creates a vulcanized seam (Malone and Laurencio 2004).

The choice of fencing material depends on the desired duration of the fences, ease of installation, availability, and funding. Funnel traps placed along drift fences increase capture rates, especially for snakes. The same commercially available minnow traps mentioned above work well for this purpose, or funnel traps can be made from mosquito screen or hardware cloth (Fritts 1988, p. 30). Most large snakes can escape pitfall traps, but not funnel traps.

Pitfall traps can be interconnected with drift fences in various configurations. Generally, researchers have assumed that fences arrayed in several directions (e.g., cross- or Y-shape) are more likely to intercept reptiles moving through a habitat than is a single straight fence. This prediction is not necessarily true, however, especially if total lengths of the fences are equal. Hobbs et al. (1994) compared drift fences of the same total length and found that more reptile individuals and species were caught when using straight fences than when using angled fences. Presumably, this occurred because a straight fence extends further from the central trap and on average traverses more territories and microhabitats. This difference can be visualized if one compares an intact straight fence with the same fence cut into many segments placed to originate from the same point. On the other hand, if herp movements are likely to be unidirectional, such as near a pond or barrier or on or near a habitat corridor, fences running in several directions or completely encircling a habitat patch (e.g., pond) may help to ensure maximum interception of animals (e.g., Pechmann et al. 1991; Dodd and Scott 1994).

Whatever the configuration, pitfall traps are always most effective if the fences are longer than 8 m, and many herpetologists use much longer fences. Initial testing may be required to determine the "best" trapping configuration and fence length for a site. For example, in an on-going study of herpetological communities, JHY and colleagues (JHY, unpubl. data) used Y-shaped arrays with total fence lengths of 15, 30, and 45 m, periodically switching the different fence-length arrays among sampling sites to remove any site effect. Following a trap-and-removal protocol and operating the traps on 14 consecutive days at 2-month intervals, they captured 590 specimens of amphibians and reptiles over 16 months. The capture efficiency was 9.5 specimens/m of fence for the 15-m array, 7.5/m for the 30-m array, and 5.0/m for the 45-m array; in other words, capture efficiency decreased with increasing drift-fence length. They tentatively concluded that for the same total length of drift fence, several widely separated arrays with short arms are more effective than fewer arrays with longer arms, presumably because of increased sampling of subtly different microhabitats within a habitat type. More arrays, however, also mean more travel time. The optimal balance of array size and geographic spread will depend upon funding, landscape configuration, and the purpose of the study. If one fence three times as long as another results in the capture of only twice as many individuals, then the longer fence actually results in the capture of proportionately fewer individuals. In that case, three more widely spaced arrays with short fences would be more efficient than an array with the longer fences, unless increased travel time significantly reduced efficiency.

Drift-fence-trap configurations may also vary according to the circumstances and goals of a study. Long fences are positioned haphazardly in suitable habitat if the objective is simply to sample a particular group of species. One of three standard configurations of pitfall traps with drift fences, referred to as "herp arrays," is commonly used in herpetological field studies. One type of array consists of four drift fences, each at least 8 m long, configured in an open cross, with 8 m separating the arms at their centers. A 20-L bucket is placed at each end of each fence and a funnel trap on each side. In a regular Y-shaped array, three fences, at least 8-m long, radiate at 120° intervals from a central pitfall (see Fig. 75, in Chapter 13). Buckets are also placed at the distal ends of the fences, which have a funnel on either side. Y-arrays use less material than other types of arrays and can be installed quickly and easily. *Minimal Y-arrays* require even less material; a single bucket is located centrally, and a single funnel is placed at the distal end of each fence. Minimal Y-arrays are advantageous at sites where pitfall buckets are prone to flooding. Placing funnel traps along drift fences increases the numbers of individuals and species captured compared to using only pitfall traps.

Pitfall and funnel trap arrays are common sampling tools in studies in which herpetological communities are compared, for example, among different treatments, in different habitats, before and after environmental interventions, and through time. It is important to note that for most analyses, the entire pitfall/funnel trap/drift fence assembly is the sample unit. A Y-shaped array, for example, is a single unit and should not be considered as four traps, because it employs four pitfalls. Standardizing the configuration used for trapping with drift fences, pitfalls, and funnel traps allows for more rigorous statistical comparisons of capture numbers.

Standardized trap/fence units can be deployed randomly within a sampling area for herpetological studies that require a statistically rigorous design. If data are to be analyzed quantitatively, then some minimum number of replicate samples must be taken within each habitat (see "Power and Effect Size," under "Precautions for Quantitative Reptile Field Studies," in Chapter 3, and "Rarefaction" in Chapter 14). Large numbers of arrays, which require significant commitment and substantial investment in materials, labor, and time, are needed for statistical rigor. Consider a simple comparison of reptile assemblages from three distinct habitats. Each habitat type (or treatment) is the sample unit of interest; replicates of the sample units must be used to provide the statistical power necessary to detect mean differences in numbers of reptiles (e.g., mean number of individuals or mean number of species) in each habitat. Furthermore, because buckets and funnels connected by drift fences constitute a single unit, arrays must be replicated in each habitat site to provide independent data points. Sampling three independent sites for each habitat type with three arrays in each site requires financing for construction and maintenance of 27 trap arrays. Depending on the configuration, 27 arrays would require from 27 to 216 buckets and at least 650 m of fence. Nine traps generally do not produce a large sample of reptiles for any given habitat; sampling additional sites in each habitat type would greatly increase statistical power and the likelihood of detecting differences.

Installing a system of herp arrays may be worth the trouble if a group is committed to the continued sampling of an area. Installing and maintaining arrays, however, is a lot of work, and capture rates will be low without a large number of arrays. Animals in traps must be protected from predation and desiccation, and traps should be checked at least every other day. The edge around pitfalls buried in the soil must be maintained, and rainwater that accumulates must be promptly removed by dipping or sponging. These tasks can be tedious, and the workload increases exponentially as additional arrays are deployed.

Nevertheless, pitfall traps and drift fences are very productive means of catching many kinds of reptiles; they are particularly important tools for capturing species that are difficult to locate with active search methods. It is common for herpetologists to set up a few fences with pitfalls and funnels even for a few days during expedition-style surveys aimed at documenting presence of species (see techniques for "Complete Species Inventories," in Chapter 13). Deploying fewer than six arrays (e.g., 24 buckets using Y-arrays) and opening them only on occasional weekends, however, does not result in sufficient captures to make it worth the trouble for studies aimed at quantifying herpetological communities; however, setting a few arrays for a few days can result in the capture of a few species that were not captured by other methods (W. Ryberg and LAF, pers. comm.). Conversely, large array systems that operate for 7 to 10 days yield large samples of reptiles. Pitfall and funnel traps must be closed securely between sampling periods, and funnel traps left inoperable, in order to prevent accidental capture of animals.

Specialized Trapping of Snakes Over Large Areas

D. Craig Rudolph

My colleagues and I (Rudolph and Burgdorf 1997; Rudolph et al. 1999; Burgdorf et al. 2005) devised a reasonably efficient trap that captures large, mobile snakes but excludes small, litter-dwelling species. The trap can be deployed over large areas, is relatively inexpensive to build, and is easy to install and maintain.

The trap consists of a 1.2-×1.2-m top and bottom constructed of 1.27-cm-thick (0.5-in) treated plywood supported by wooden uprights 0.45 m tall (Fig. 22). The sides are made of hardware cloth (3.2-mm mesh). A hardware-cloth funnel with an inner opening approximately 4 cm in diameter is wired over an opening at the center of each side; snakes enter through the other end of the funnel, which rests on the ground. One or two hinged doors in the top provide investigator access. We placed traps on the surface of soil that we had leveled and filled to eliminate gaps beneath them. We then constructed drift fences 15 m long by 60 cm high from 3.2-mm-mesh hardware cloth. Sediment cloth, metal flashing, and other materials can also be used. A vertical drift fence was installed on each side of the trap at the midpoint of the funnel entrance. The fences were buried to a depth of ca. 10 cm and braced with wooden stakes or short pieces of iron reinforcement bar (rebar) as required. Mesh size, height, and length of drift fences can be varied to accommodate animals of different sizes.

Each trap was supplied with a 1-gal passive water supply (chick waterer) and covered with a limited amount of soil and debris. If possible, traps were placed at sites with some shade to reduce the likelihood of heat stress in captive individuals. Researchers working in open habitats may have to provide artificial sources of shade. Because of these accoutrements and the relatively large size of the traps (essentially a rather spacious cage), we only had to check the traps to remove captured animals once per week.

Trap success varied with site characteristics. In upland pine habitats in eastern Texas where most of our work was done, we caught 6 to12 snakes per trap array per season (early March to late October; 240 trap-days), depending on the site. Most individuals captured measured in excess of 30 cm SVL. This is

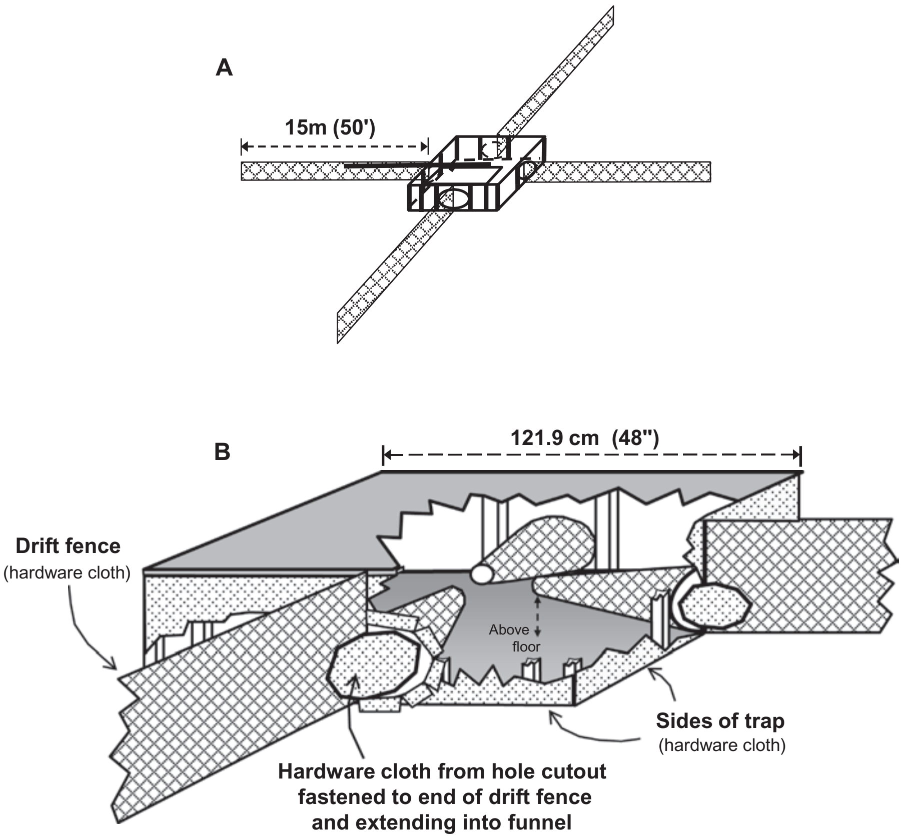

FIGURE 22 Schematic of trap designed to capture large, mobile snakes. (A) Trap and drift fence array. (B) Detail of trap and drift fence attachment. (Reprinted, with permission, from Burgdorf et al. 2005.)

an acceptable capture rate for upland habitats fragmented by a dense network of roads. We captured few small, litter-dwelling species of snakes, presumably because they were reluctant to leave the litter and ascend the inclined funnel to enter the trap, and because individuals could pass through the fairly large openings in the mesh. Funnel orientation and mesh size can easily be adjusted, however, for use with other species.

With this protocol, mortality due to stress was low, usually less than 2 percent. However, infestations of nonnative fire ants (*Solenopsis invicta*), which killed the snakes, were a serious problem. To control the ants within the traps, we treated all visible mounds within 10 m of a trap with a variety of commercially available ant poisons. We also suspect that ophiophagous Common Kingsnakes (*Lampropeltis getula*) occasionally entered traps and ate other captured snakes.

We captured a variety of animals in the traps other than snakes. These generally presented few problems, but birds and small mammals frequently entered the traps and died. Birds, in particular, tended to injure themselves attempting to escape. Reducing the diameter of the outer funnel entrance to 3 to 4 cm, prevented most birds from entering the traps and had no obvious impact on capture rates for snakes.

Because we needed to visit traps only once per week, we were able to survey a large area. With traps dispersed over hundreds of square miles, their frequent checking rapidly becomes cost and time prohibitive. Depending on particular needs, one must assess the trade-offs between costs, labor, logistics, and capture rates associated with a few large traps and many small traps.

Special techniques are required for the safe capture and handling of venomous snakes. The first admonition is that no one should ever try to catch a venomous snake without a compelling reason. Pitvipers can often be identified and photographed *in situ*; if undisturbed, they often stay put for an extended period. Biologists monitoring herpetological communities should have detailed information on all venomous snake species that they are likely to encounter, and they must be trained to identify, capture, and handle them. The project safety and evacuation plan for any field research project should also include a protocol addressing snakebite (see “Handling Live Reptiles,” in Chapter 8, for additional information on handling venomous reptiles).

Finding, Counting, and Catching Crocodiles

Frank J. Mazzotti

With few exceptions, crocodilians must be counted and caught for studies of their ecology. Most crocodile biologists use harvesting methods (Chabreck 1965; Jones 1966) that have been modified to suit a particular target species or habitat and to minimize risk of injury to both the crocodile and the investigator (Webb and Messel 1977; Hutton et al.1987; Walsh 1987; McDaniel and Hord 1990). It is likely that each situation will require its own adaptations or modifications of methods used by others.

The extraordinary range of body sizes of crocodilians presents a challenge to those catching and handling them. Naive hatchlings, about 25-cm long and weighing about 25 g, can easily be grabbed by hand. Large adults, which can reach 5 m in length and weigh up to 500 kg, are wary and unapproachable.

Once captured, crocodilians must be restrained for processing and marking. Removing restraints from and releasing a large crocodile are the most dangerous aspects of an already hazardous task.

Finding Crocodilians

Techniques used to find crocodilians depend on habitat, cost, field logistics, and goals of the study. Occasionally, crocodiles can be approached by automobile or encountered during a walking survey. Because crocodilians live in aquatic environments, however, some type of watercraft is usually required. Fixed-wing aircraft and helicopters can be used to find crocodilians in areas not accessible to boats or when large areas must be covered quickly. Aerial surveys are especially effective in relatively open habitats for large-scale surveys for distribution and abundance of crocodilians or their nests, but they are not as effective in closed habitats (Magnusson 1982; O'Brien 1990). Small-scale surveys and detailed studies of demography and habitat use are usually done from boats.

Watercraft used to find crocodilians commonly have a shallow draft and an open interior. They include kayaks, jon boats, canoes, pirogues, marsh skiffs, fishing skiffs of various design, and airboats and are propelled by paddle, pole, or small, portable outboard or inboard engines (or electric motors). Fishing skiffs can be powered by larger outboard engines, which are sometimes permanently attached. Traditional outboard motors work well in open water. A special engine made by Go-Devil Manufacturers of Louisiana (Appendix II) is reportedly effective in areas with dense vegetation, mud, stumps, and rocks. Fishing skiffs can also be "poled" over areas that are too shallow for an engine. Airboats have a very shallow draft and flat-bottomed hulls made of aluminum or fiberglass. Propellers, similar to those on aircraft, are mounted in a cage in the stern of the boat and powered by aircraft or automobile engines, although the former are more effective because they have a better horsepower-to-weight ratio. Specially designed airboats can run on little or no water.

Seasonal wetlands may dry out periodically leaving crocodilians concentrated at isolated ponds and pools, in which case some form of all-terrain transportation is needed. In the Venezuelan llanos, investigators generally visit caiman ponds on horseback, although four-wheel-drive all-terrain vehicles can also be used. In Florida a variety of custom-built "swamp-buggies" are used when the ground is exceptionally soft or rocky or the water deep. An all-terrain amphibious vehicle (Fig. 23) can be used as a wheeled- or tracked-vehicle and is also capable of crossing deep water such as that in canals. With tracks in place, the vehicle has a soil impact pressure of less than 60 g/cm^2. This is less than the impact of a human footstep and minimizes damage in environmentally sensitive areas.

Wandering in and finding the way out of a swamp makes the ability to navigate with map and compass a must. Global Positioning System (GPS) receivers are helpful, but they do not replace basic orienting skills. GPSs also allow investigators to reference locations of sightings, captures, environmental data, and other habitat features accurately for later analysis.

Crocodilians are more active at night, and their eyeshine (light reflected from the *tapetum lucidum*) is visible. Consequently, night eyeshine or spotlight surveys are the preferred methods for finding, counting, or catching these animals (Magnusson 1982), even though working at night exacerbates the difficulties of catching large biting animals from small boats. Good lights make a big difference. Halogen and xenon bulbs produce a very bright light. Marine and dive lights are especially suitable because they can withstand wet conditions. If effective spotlights are not available commercially, they can be fashioned from automobile headlights and batteries. Brighter is not always better, however, because very bright lights wash out eyeshine from nearby crocodilians. Lights in the 50,000 to 200,000 candlepower range seem to be the most effective, and blue beams have much less glare than white lights. Headlamps are as important to a crocodile catcher as spotlights. They are essential when a watercraft is propelled manually and during capture events when one's hands must be free. Many types of headlamps have been designed for mining, hunting, and camping purposes (see "General Products for Field Research" in Appendix II).

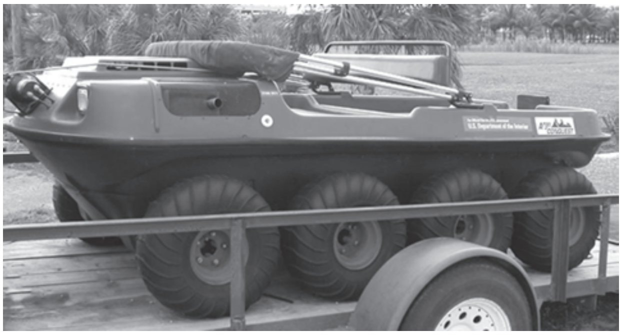

FIGURE 23 All-terrain amphibious vehicle that can be used in deep water and to traverse large expanses of dry areas between wet ones. (Photo courtesy of Michael S. Cherkiss.)

Factors other than population density (e.g., air and water temperatures, wind, wave action; Woodward and Marion 1979) affect the number of crocodilians located during a survey, and not all of the crocodilians in an area will be counted (Graham and Bell 1969; Magnusson et al. 1978; Bayliss 1987). Habitat type (e.g., pond or marsh) and accessibility also influence the proportion of crocodilians observed, as do the skill of the observer and the types of boat, motor (if any), and light used. Results obtained by two observers shining their lights and counting independently can be used to estimate the numbers of presumably visible crocodilians that have been missed (i.e., that have been seen by only one observer) during surveys (Magnusson et al. 1978; Bayliss 1987). This does not account for crocodilians not seen because they were submerged or obscured by vegetation or some other habitat feature. Wariness of crocodilians can be estimated with the distance of an approaching observer from a crocodile when it submergences (Webb and Messel 1979; Pacheco 1996). Visibility in different habitats can be tested by thoroughly searching a sample area after a typical survey or by having one crew member set out dummy crocodilians to be counted by a survey crew.

Catching Crocodilians

Hunting and trapping are the two basic approaches to catching crocodilians. Whether to hunt or trap and the technique of hunting or trapping to use depend on many factors, including size, wariness, and hide toughness of the target species; the habitat they occupy; the mode of transportation used by

THIS PART SNAPS ABRUPTLY LIKE THIS, LOCKING THE GATOR IN THE TRAP SO ITS UNABLE TO ESCAPE!
USTACH 2010

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the investigator; and the fate of the animal. If a crocodilian is to be killed after capture, then methods that may cause mortal wounds can be employed. Because most biodiversity studies involve live animals, however, we focus, with few exceptions (e.g., harvest harpoons and baited hooks), on methods designed for the safety of the crocodile and the investigator.

The advantages of hunting crocodilians are that the researcher can select which animals to catch and minimize handling time. The advantage of trapping is the potential to catch animals too wary for humans to approach. Trapping is not, however, selective, and trapped animals are at risk of injury from struggling, drowning, overheating, or attack (Walsh 1987; Leslie 1997). Checking traps at appropriate intervals is very important. Traps can be rigged with transmitters that are activated when an animal moves, tripping the securing device (Webb and Messel 1977).

If a crocodile regularly uses a habitat feature such as a trail, nest site, basking site, or den, then a walk-through (or swim-through) trap can be set to catch the animal. Bait can also be used to attract a crocodilian to a securing device; it can be attached to a triggering device to hasten the closing of the trap (Murphy and Fendley 1974; Leslie 1997). Counterweights, leaf springs, bamboo and fiberglass poles, and rubber tubing have all been used to spring traps (Pitman 1941; Murphy and Fendley 1974; Webb and Messel 1977; Hutton et al. 1987; Walsh 1987). On the other hand, nontarget animals may eat the bait, leaving the trap without an attractant and either activating or disabling the triggering device.

Large baited hooks set over a body of water are effective for catching crocodilians to be killed, although if digestible hooks are used, captured crocodilians can be released. A digestible baited hook is made by running a natural fiber rope (hemp or cotton) through a hollow bird bone (Walsh 1987) or wooden dowel and then surrounding it with a chicken carcass or a pig or cow lung. When the bait is swallowed, the dowel or bone becomes lodged inside the stomach, effectively "hooking" the crocodilian. The rope can be cut after the animal is retrieved, and the trapping apparatus is then digested.

A noose can also be baited; when it closes, it catches the crocodile around one jaw, which increases the risk of injury but works well with heavy-jawed species (Kofron 1989). Nooses, nets, boxes, or cylinders can be used as securing devices with triggered, baited traps. Box and cylinder traps are unwieldy, however, and crocodilians readily become trap shy (Leslie 1997) and can easily hurt themselves while struggling (Walsh 1987). Nooses and nets are effective for large crocodilians, cause minimal injury, and are reusable (Pitman 1941; Murphy and Fendley 1974; Webb and Messel 1977; McDaniel and Hord 1990; Leslie 1997).

Walk-through snares can be set on trails (Mazzotti and Brandt 1988; Wilkinson 1994, fig. 3), and floating nets can be set across creeks or in ponds (Webb and Messel 1977). Fish may get caught in the net and act as bait. Floating nets need to be monitored almost continuously so that the crocodilians do not drown. Crocodilians in dens or caves can be trapped by setting nooses or nets at the exit. Sometimes the underground path of a crocodilian can be followed by probing into the soil surface with a metal rod. An investigator can then prod the animal, encouraging it to leave the den. Digging down into the den to expose the crocodile is also possible, but we do not recommend it, because it destroys a critical habitat feature on which other aquatic animals may depend.

The traps described above were designed for large, wary crocodilians and are not as effective for smaller animals. A basking trap (modified turtle trap), in contrast, is effective for small, wary crocodilians (Petokas and Alexander 1979). The investigator builds a floating platform, places it in an area where small crocodilians have been observed basking, and surrounds it with nets. Once crocodilians occupy the platform, she or he approaches and startles them so that they dive off the platform into the nets. Blocking or removing alternative basking sites encourages the crocodilians to use the platform.

Camera trapping does not involve animal handling (Thorbjarnarson et al. 2000). Optical or digital cameras are set in areas of activity (nest sites are particularly productive), and the camera is triggered by heat or motion sensors. The picture is date- and time-stamped.

Small crocodilians (<1 m total length) can be captured by hand or with tongs, nooses, and small weighted treble hooks, as well as with dipnets, cast nets, seines, and other nets. Crocodilians larger than 1.25 m (or even 1 m) can struggle free from hands and tongs and bite an investigator. These animals must be plucked quickly from the water and placed in the boat or pinned to the substrate to prevent the escape of or injury to the animal (Webb and Messel 1977). Larger alligators and crocodilians can be caught with large nooses (self-locking wire nooses are best), small harpoons, small treble hooks, and nets (see Appendix II).

Nooses work well if one can approach an animal close enough to place a noose around its neck (Chabreck 1965; Jones 1966; Mazzotti 1983; McDaniel and Hord 1990). The noose is made of good quality line, which is then attached to a float or to the catch boat. The noose is also attached to a noose pole which facilitates its placement. Nooses are set by pulling or jerking. Pullers hold the line, and when the noose is around the alligator's neck, they pull the line quickly, closing the noose. In jerking, the researcher jerks the pole in a manner similar to setting a hook during fishing. Poles can be made from wood, bamboo, fiberglass, plastic, and other materials, depending on preference and availability. Poles should be stiff and lightweight; those used for jerking should have a certain amount of flexibility. Telescoping poles facilitate transport. For small alligators, the noose is firmly attached to the noose pole. For larger alligators, it is best to attach the noose with tape or spring clips so that it can break free from the pole (which becomes unwieldy as the alligator thrashes), allowing the alligator to tire itself out before it is hauled on board. Lines should be attached to floats when catching large crocodilians from small boats, so that the whole rig can be thrown into the water if a fighting crocodile threatens to upset the boat. The animal can be retrieved later.

Darts are harvest harpoons with the penetrating head reduced so that the depth of penetration and the likelihood of injury to the animal are lessened (Jones 1966; Webb and Messel 1977; McDaniel and Hord 1990). Darts are attached to the end of a pole that is thrust by hand. They are also attached to a line and can separate from the pole. Darts should be placed away from internal organs in areas of relatively soft hide, such as the jowls and the sides of the tail. This can be tricky, particularly if the animal is diving; also, the dart must be delivered with enough force to penetrate the hide. Short punching strokes provide the best combination of power and accuracy. Although we have successfully darted animals as small as 1 m total length, the safe target area on animals less than 1.5 m long is so small that the risk of injury increases dramatically. Because darts can pull free when an animal struggles, they are used primarily to keep a line on the crocodile until it can be noosed.

Larger hand-thrust harpoons can be used in harvest situations, as can propelling devices. Hunting bows and spear guns are popular methods of delivering harpoon heads. In catch-and-release situations, it is difficult to propel a dart accurately with enough power to penetrate the hide and not cause injury. Weighted treble hooks cast out beyond a crocodile can snag the animal during retrieval (McDaniel and Hord 1990). Crocodilians in ponds that are otherwise inaccessible can be snagged with treble hooks dangled from a maneuvering helicopter. The catcher then jumps into the pond and tows the crocodile to the nearest shore to be processed. Since hooks can pull free and injuries can occur when a crocodile struggles, the animal should also be noosed as soon as it is within reach.

Crocodilians less than 1.2 m total length can be caught with dipnets. The net is placed in line with the handle for scooping (Jones 1966) or at an angle to the handle for pressing down over the alligator from above (Webb and Messel 1977). Cast and seine nets are effective in open water but readily catch on vegetation and rocks.

Crocodilians up to 1.5 m can be brought into a boat. The mouths of crocodilians ≥0.5 m total length should be secured shut before handling to prevent them from biting the investigator or grabbing hard objects that may break their teeth. Rope, rope or wire nooses, plastic cable ties, and rubber bands can be used to shut the mouth, and duct or electrical tape to secure it closed. The mouths of alligators, which can be particularly difficult to shut because closing devices slip off their smooth, rounded snouts, and other large animals can be secured with heavy-duty rubber bands or strips of inner tube with pieces of line tied to them. After release, when the crocodilian is some distance from the captor, the elastic can be removed by tugging on the line. A crocodilian with its mouth secured should never be freed or allowed to escape.

Once the mouth is shut, a crocodilian can be further secured by tying or taping its legs together dorsally (for short periods only), covering its eyes and ears, and if necessary, fastening it to a board or other suitable hard object. No safe method for chemical capture of crocodilians is known; however, animals can be immobilized for measurement, translocation, and surgery (Loveridge and Blake 1987). The muscle relaxant Flaxedil (gallamine triethiodide) has been used successfully on *Crocodylus niloticus* (Loveridge and Blake 1987; Leslie 1997) and *C. porosus* (Walsh 1987), although drug suitability and dosages are likely to differ among species. A pole syringe is the simplest and most effective means of delivering an intramuscular injection. Neostigmine methyl sulfate is the antidote for Flaxedil (Loveridge and Blake 1987). The greatest danger from immobilizing drugs is their accidental injection into the person administering the shot (Buys 1973). A protocol for emergency treatment after accidental injection should be part of any immobilizing procedure.

Animals that are captured, quickly processed, and released show few ill effects from handling. Crocodilians that have struggled in a trap for any length of time or been immobilized need a recovery period. Loveridge and Blake (1987) emphasized that during recovery from Flaxedil, crocodilians must be placed in shallow water to prevent drowning. Crocodilians that have been handled after trapping should be allowed to recover on their own, next to the water. Crocodilians should not be allowed to get too hot or too cold during handling and recovery.

Catching large crocodilians at night, while maneuvering in shallow water often strewn with obstacles, is a potentially dangerous activity. With due care and caution, however, both crocodilians and researchers can emerge unharmed.

Collaboration with Local People for Sampling Reptiles

Marcio Martins and Cristiano Nogueira

Availing yourself of help from local people in finding reptiles can be a productive means of documenting species in a study area and increasing samples of targeted species. Furthermore, local people can be asked to capture or kill the squamates they find, providing a valuable source of scientific specimens for surveys as well as for studies of populations and communities (Cunha and Nascimento 1978; Marques 1998). Fitzgerald et al. (1991) employed hunters to help find and capture tegu lizards

FIGURE 24 Aguaruna men bringing in specimens as part of a biodiversity survey of the surrounding forest. The men on the left have prepared their catch as study skins. The bags carried by the man on the right contain live reptiles, including the only specimen of *Uracentron flaviceps* observed during the study. Village of Huampami, Río Cenepa, Department of Amazonas, Peru, July 1977. (Photos courtesy of M. S. Foster.)

(*Tupinambis merianae* and *T. rufescens*) in Argentina and used carcasses of hunted tegus as vouchers and for information on diet and reproductive biology (Fitzgerald et al. 1993). Local people in the areas where we have worked learned the importance of specimens for scientific research quickly and have been amenable to assisting with research projects (MM and CN, pers. observ.; Fig. 24).

Local people can store dead squamates if provided with a 20- to 30-L plastic container half-filled with 10 percent buffered formalin. Preserved specimens can be kept for long periods, although monthly or bimonthly visits yield natural history data from specimens that can be analyzed by time period. Training collectors to make short incisions on the venter of large specimens may prevent putrefaction. Local collectors can also inject formalin into the body cavity and limbs of recently killed specimens, provided that they receive proper instruction and materials. Containers and collecting materials can be distributed across the area to be sampled, in accordance with the need for specimens and the number of local inhabitants.

Often, local assistants can be taught to fill in short forms provided by the researcher, noting (at least) the date of capture, time of day, and habitat. Forms and specimens can be linked if numbered labels are provided and attached to the animals. In practice, however, it is not easy to convince people to be careful with field data and labels, although some local collectors show interest in the animals and procedures and become good sources of well-preserved material and reliable information. In the course of a study, poor collectors can be identified and replaced.

Another way to obtain field data is to ask each collector for details about each specimen he or she has collected. In this case, researchers should retrieve specimens from collectors at least every month, which will also verify the month of collection, which is useful for studies of activity and reproduction. Distant or inaccessible collecting sites need be visited only every 2 to 6 months, provided that each preserved specimen is correctly labeled and preserved. Thus, specimens can be obtained even from remote localities (especially relevant for diversity surveys), although it may be harder to obtain accurate ecological data. Local collectors must be instructed to capture squamates of all kinds and sizes, although a bias toward large, perianthropic, and/or slow moving species is virtually unavoidable. The researcher should also provide careful instructions for avoiding bites of venomous snakes.

Specimens obtained from local collectors can make a useful contribution to field studies of local squamate diversity, especially those with a large geographic scope. The data obtained from disturbed areas sampled by local collectors can be combined with those obtained by the researcher from undisturbed sites (using diverse sampling methods) to produce a reliable survey of local squamate communities. Furthermore, researchers conducting studies in undisturbed habitats that are surrounded by disturbed areas can infer which native species tolerate disturbance and determine the extent to which nonnative species have invaded the region. Specimens preserved by local people just after being collected can be useful sources of dietary, reproductive, and morphological data. On the other hand, specimens are not generally preserved carefully and may have been damaged (especially snakes) when collected. Even so, most reptiles are still good for scale counts and as a source of ecological data. When collecting sites are easily accessible and frequent visits are possible, properly trained local collectors can obtain and maintain live squamates. Animals provided with water and kept in wood, polystyrene, or glass containers (provided by the researcher) in cool, shady places can live for several weeks. In most countries killing and/or keeping live native animals requires a permit; the researcher should obtain proper permits before initiating the study.

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We thank Mercedes Foster for her careful editing, and for seeing the book project through to completion despite many hurdles. We also acknowledge the work of herpetologists everywhere who developed the techniques we described, and who have, both formally and informally, shared their expertise in how to find and capture reptiles.

Contributors

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CHAPTER SIX

Voucher Specimens

ROBERT P. REYNOLDS and ROY W. McDIARMID

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Introduction

A basic requirement of all scientific inquiry is that other investigators be able to repeat a study. They must duplicate the conditions under which an experiment was carried out or an observation was made; in biological studies that means that the study organism must also be the same. That is why scientific names of subject organisms are always reported instead of (or in addition to) categorical or common names (e.g., rats). As we learn more about the taxonomy of organisms and their phylogenetic relationships, however, scientific names of species may change, and single species may be shown to encompass previously unrecognized species clusters. In addition, errors in identification can occur under any circumstances, especially among hard-to-recognize forms (e.g., sibling species). To allow for the future verification of the identity of study organisms, whether used in laboratory experiments or observed in the wild, biologists collect and prepare what are known as *voucher specimens*. Voucher specimens provide the means whereby a researcher can verify the identities of organisms encountered or used in a previous study. Other investigators can consult them to verify that identity and thereby ensure the accuracy, consistency, and repeatability of any subsequent study.

Confirmation of published identifications through the examination of voucher specimens is the only mechanism for validating statements about the presence of a species at a site and for making historical comparisons. Lee et al. (1982) provided a cogent review of voucher specimens and their importance to biological studies, and we have adopted many of their recommendations in this presentation. Despite the importance of vouchers, however, their deposition is still the exception rather than the rule, especially in nontaxonomic studies, and it is rarely required by editors, reviewers, and publishers of scientific papers (Bortolus 2008). In addition to their importance in systematics studies and for documenting global biodiversity through biotic surveys, properly prepared specimens deposited in museum collections can provide irreplaceable data on morphology, phenology, biochemical properties, demographic trends, geographic distributions of organisms, and other topics, and they are critical for anticipated (e.g., consequences of global warming, impact of invasive species) as well as yet unimagined future investigations (Cotterill 1995; Suarez and Tsutsui 2004). A relatively recent but increasingly important use of reptile specimens is in the study of forensic herpetology, the application of science to the study of amphibians and reptiles when these animals become the subject of legal investigations (Baker 2008). The types of legislation involving forensic herpetology are diverse and include, among others, conservation, trade, import and export, animal welfare, theft, keeping animals in captivity, human health, and damage to property (Cooper 2008).

Voucher specimens provide scientific credibility to inventory and monitoring projects and should be collected unless a compelling reason not to do so exists. Reasonable arguments for not collecting voucher specimens include legal prohibition, endangered or threatened status of the species, high risk of detrimental impact to a population from loss of a single individual, and the like. Traditional voucher specimens of reptiles are formalin-fixed and alcohol-preserved whole adult specimens (Fig. 25); for larger species, such as crocodilians, some snakes, and some lizards, separate skins, skeletons, and/or skulls (Fig. 26) may be preserved. For turtles and tortoises, shells or soft parts may suffice. The value of a specimen that serves as a voucher is greatly enhanced if it is accompanied by ancillary material such as samples of ectoparasites and endoparasites, series of good-quality photographs, and tissue samples for molecular analyses. In a few situations, tissues themselves (a clipped digit or shed skin) or photographs of an individual may serve as a voucher. However, the utility of

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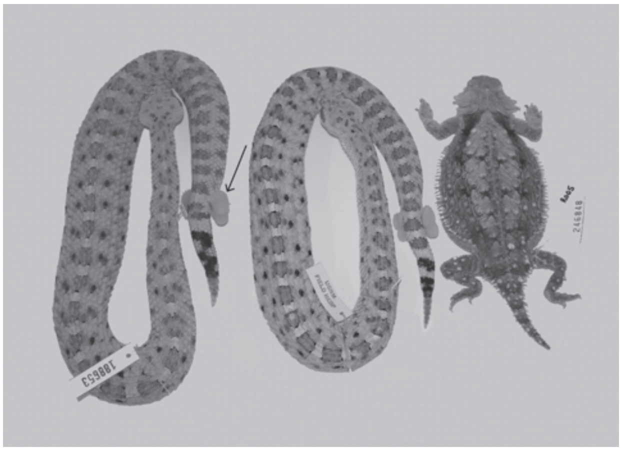

FIGURE 25 Voucher specimens of Sidewinders (*Crotalus cerastes*) and a Regal Horned Lizard (*Phrynosoma solare*) from Arizona cataloged at the U.S. National Museum of Natural History. Note USNM numbered field tags attached to the specimens and everted hemipenes of the snake (arrow). (Photo by R. P. Reynolds, U.S. Geological Survey.)

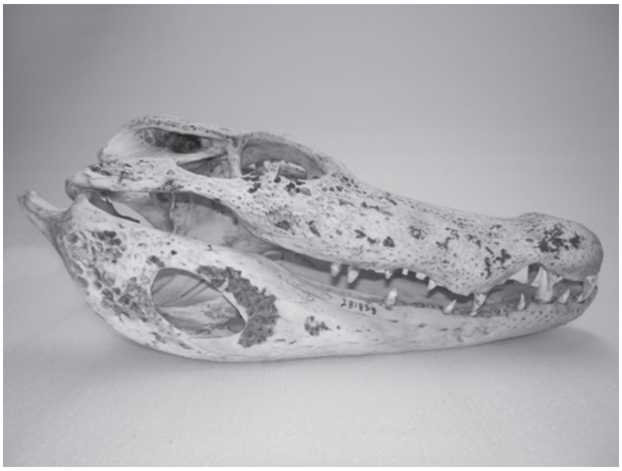

FIGURE 26 Voucher specimen of *Caiman yacare* cataloged at the U.S. National Museum of Natural History (USNM). Complete skull salvaged from Lago Tumi Chucua, Department of Beni, Bolivia. Note the USNM number written directly on the specimen with permanent ink. A numbered field tag is also attached to the specimen (Photo by R. P. Reynolds, U.S. Geological Survey.)

secondary samples (blood, skin) or indirect representations of a specimen (photograph, recorded vocalizations) is limited; they offer only a fraction of the information afforded by whole-body scientific specimens, both initially and in the long term (Dubois and Nemésio 2007). For example, they will rarely yield additional information (e.g., reproductive condition, food habits) in the future and are not very useful in comparative studies of any attributes except, perhaps, DNA. To fulfill its function, an ideal voucher specimen, in addition to exhibiting the recognized diagnostic traits of the species, should be properly preserved by the collector (see "Preparing Scientific Specimens," in Chapter 7), documented with appropriate field data, deposited and maintained in a suitable institution, and readily accessible to other investigators (Lee et al. 1982; Huber 1998).

Whenever possible, photographs ("Preparing Scientific Specimens," in Chapter 7), tissue samples (see "Collecting and Preserving Tissues for Biochemical Analyses," in Chapter 7), and parasites ("Field Parasitology Techniques for Reptile Surveys," in Chapter 7) should be taken from specimens being prepared as vouchers. Although such items are not strictly required for inventory and monitoring projects and normally cannot substitute for well-preserved scientific specimens, it is important to take advantage of opportunities to obtain such materials. Photographs provide details of color in life for many taxa that fade or change color in preservative, and tissue samples can be analyzed to estimate genetic relatedness, resolve systematic relationships, and reveal cryptic species. These ancillary materials increase the information available for each species at a site and may reduce the need to collect similar material from additional specimens in the future. However, taking photographs and tissue samples and preserving parasites often require additional equipment and supplies and a significant expenditure of field time, so an investigator must plan for these activities before a study is initiated to avoid compromising the goals of the inventory or monitoring study.

The increased interest in and value of molecular systematics have also highlighted the importance of taxonomic voucher specimens in scientific investigation. Gene-sequence data alone, even when deposited in an electronic database such as GenBank (U.S. National Institutes of Health database of DNA sequences), do not constitute a suitable voucher specimen because species-specific morphological characters cannot be examined without a permanently preserved traditional specimen and, therefore, species identification cannot be verified. Unfortunately, GenBank does not require that deposited sequences be documented with voucher specimens, and numerous examples of gene sequences in GenBank have been taken from misidentified specimens and thus, unknown species (Ruedas et al. 2000; Harris 2003). Only by reference to a voucher specimen from which the genetic tissues were obtained can the identity of a study organism be confirmed and the results and conclusions of a study seriously reevaluated. In addition, vouchers allow one to determine if unexpected results are of biological interest or merely the result of species misidentifications (Funk et al. 2005). We strongly encourage all researchers to collect voucher specimens and associate them with tissue samples and with sequence data deposited in GenBank. In the absence of vouchers, one can legitimately question whether a study was scientifically grounded.

Field Identification

Accurate identification of reptile species in the field is often difficult, especially in tropical regions characterized by high species diversity. Even in well-studied areas, identification of certain species can be challenging because diagnostic characters are often subtle and difficult to see without magnification and/or dissection, and few biologists are able to identify all species at a field site. Accordingly, we recommend that all field identifications made during faunal inventories in little-studied regions be treated as tentative and that all species be considered of equal importance when sampling.

Except for well-studied faunas as occur in sites in North America and Europe, few useful field guides or identification manuals with reliable and authoritative keys for reptiles exist, and for many countries a comprehensive list of recorded species either is not available or is seriously outdated. We strongly recommend that investigators become familiar with available field guides and the primary literature before commencing an inventory and, whenever possible, examine preserved specimens of species from the area of interest prior to beginning

YOU'RE A BURRO'S ASS?!
NO, I'M A BURROWING ASP!
A WHAT?
ATRACTASPID!
USTACH 2010

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fieldwork. Notes on the reptile fauna of the region along with a list of the species and their diagnostic features should allow an investigator to identify the more common species at a site, focus on those of specific interest, and recognize any taxa that may be protected (see "Permits" under "Collecting and Preserving Tissues for Biochemical Analyses" in Chapter 7).

Many of the older monographs on reptile faunas (e.g., Pope 1935; Deraniyagala 1939; Taylor 1953, 1956, 1963; Alvarez del Toro 1960) were based almost entirely on (often poorly) preserved museum specimens and are of limited utility for field identifications or as sources of general information on geographic and habitat distributions. Fortunately, several excellent treatments of the reptiles of particular areas have appeared in the last decade or so. References to many of these are provided in Chapter 2, "Reptile Diversity and Natural History: An Overview," and we will not duplicate them here. There are also a few additional references of interest: for Baja California and Cohuila, Mexico, see Grismer 2002 and Lemos-Espinal and Smith 2007, respectively; for the Honduran Mosquitia, see McCranie et al. 2006; for La Selva and the Caribbean Slope of Costa Rica, see Guyer and Donnelly 2005; for Peru, see Lehr 2002; and for Egypt, see Saleh 1997. Guides to various other areas are in preparation.

Herpetologists, even those with considerable experience in an area, often record only generic or tentative specific identifications of specimens in their field notes. Such names serve for bookkeeping purposes and are useful for tracking the numbers of species and specimens sampled during an inventory project. Upon returning from the field, and prior to publication of any data collected during the project, all field identifications should be verified by a person experienced with the taxonomic group or by comparison with correctly identified specimens in museum collections. Erroneous field identifications may cause investigators to overlook specimens of poorly known species or ignore important data because they assume that the species involved is well known. The literature is replete with examples of comparative studies in physiology, ecology, behavior, morphology, and systematics for which research results are questionable (or even useless) because study animals are likely to have been misidentified, an absence of vouchers precludes verification, and/or the study 'species' is now known to encompass several distinct taxa. Most decisions relating to the management and conservation of species also depend on accurate species identifications.

The importance of voucher materials cannot be overestimated, and the proper preparation of such specimens is essential for good science. We acknowledge, however, that the removal and preservation of specimens for scientific purposes can be an emotional issue. Therefore, it is essential that field investigators carefully plan their studies in advance, clearly identify the study objectives, evaluate the need to collect voucher specimens, and maximize the amount of field data gathered for each voucher prepared. It is also essential that governmental and nongovernmental agencies requiring or supporting biodiversity assessments acknowledge the critical need for voucher specimens, authorize their collection, and provide financial support for their preservation in the field and maintenance in museums.

Sample Size

The number of vouchers specimens collected during a biotic survey is largely determined by the nature, demands, and duration of the project and the experience and thoroughness of the investigators. Limited space and personnel, together with costs of accessioning, identifying, cataloguing, managing, and storing natural history specimens, often affect the number of specimens that a research museum can or is willing to accept from large-scale studies, particularly inventories. It is therefore important for investigators to consult during the planning stages of a study with the curatorial staff of the research museum that will likely be the repository of the samples. They can obtain an estimate of the number of voucher specimens that the museum can or is willing to accommodate, as well as the institution's requirements regarding specimen preparation, data documentation, and so forth.

The size of an adequate or optimal sample for the purposes of identification is not easily determined. Fewer specimens generally are needed to document species occurrences in well-studied areas in North America, for example, than from remote regions in the Amazon Basin of South America or the Congo Basin of Africa. Some species can be identified from a single specimen; others are so variable that 20 individuals would not adequately represent the population, and a larger sample would be necessary. Some species are polymorphic (e.g., *Corallus hortulanus*), and in some species individuals may vary even within the same litter (*Atropoides mexicanus*). Other species exhibit striking sexual (*Graptemys barbouri*), ontogenetic (*Sphaerodactylus* sp.), and/or geographic (*Drymarchon* sp., *Platemys platycephala*) variation. In contrast, some reptiles (e.g., *Oxybelis aeneus*) are relatively uniform even across broad geographic areas (see also "Identification and Natural History," under "Know Your Organisms," in Chapter 3). Modern systematics takes into account the potential for morphological variation and uses other biological data (e.g., genetic information) to understand species limits. Gone are the days of running a single specimen through a key and magically achieving a reliable specific identification. That "cookbook" approach is scientifically unsound; in our experience a single specimen from a site is rarely representative of an entire species. Properly constructed keys can be useful tools for obtaining preliminary identifications, but to ensure reliability such identifications must be verified by comparisons with descriptions in the literature and with preserved museum specimens from nearby locations. On a global scale, the quality and availability of keys varies widely; even in the United States, keys for identifying reptiles from poorly studied areas are inadequate or nonexistent.

We agree with Frith (1973, p. 3) that the number of animals sampled "has no [biological] significance unless it is related to the total number of animals in the population and their rate of replacement." Concerned readers will find a cogent discussion in Ehmann and Cogger (1985, especially table 3) of what many consider an unwarranted preoccupation with survival of individuals, as well as quantitative data on the relative impacts of scientific collecting, natural mortality, habitat destruction, and commercial collecting on reptile populations. It is revealing that not a single species of animal is known to have been exterminated as a result of scientific collecting during the 250-year history of systematics research (Hedges and Thomas 1991; Dubois and Nemésio 2007). In contrast, hundreds to thousands of species have likely gone extinct as a result of habitat destruction (Wilson 1992). With few exceptions, reptiles are prolific, with reproductive potentials sufficient to accommodate increased levels of predation. As predators on reptiles, scientists usually are singularly inefficient compared to snakes, birds, and other mammals. Furthermore, preparing specimens and recording the data associated with them (see "Preparing Scientific Specimens," in Chapter 7) are time-consuming activities, when done correctly, which tends to discourage random oversampling (see also Foster 1982; Ehmann and Cogger 1985).

It would be convenient if we could provide an absolute value for (or formula to calculate) the number of vouchers of a given species that should be collected, but science is rarely convenient. Providing a meaningful formula for each of the more than 9,000 species of reptiles is beyond our ability. For an inventory in a region where the reptile fauna is well known, the minimum number of vouchers that will suffice is a single adult specimen of each sex of each species at each sampling site. As an operational figure, 10 to 20 adult specimens (males and females) should adequately represent a species at each collecting site in well-studied areas. If larger numbers of specimens are collected, positively identified specimens can be deposited in multiple repositories, facilitating access by many researchers. For monitoring studies, we recommend that the investigator preserve a voucher at the initiation of the study. Normally, the first adult of every species encountered during a project is collected. If additional vouchers are required, they can be taken at the end of the study or from areas adjacent to the study site.

Because we are in an early discovery phase and do not understand the taxonomic relationships of many tropical reptiles, and because many tropical areas are poorly known (Fig. 27) and numerous species are likely undescribed or inadequately represented in systematic collections, we recommend that investigators in such areas collect many more than two (one male, one female) voucher specimens per species. Generally speaking (and recognizing the limitations of any generalization), we recommend that 25 individuals (ideally 10 adult males, 10 adult females, and 5 immatures) be collected at poorly known sites. Sample sizes also must be sufficient to permit statistical analyses and comparisons. The conventional but arbitrary significance level of $\alpha > 0.05$ in statistical analyses for rejecting a null hypothesis suggests that a reasonable minimum sample for each species from a locality would be 20 individuals per age and sex class. In practice, however, samples of this size can rarely be obtained for a species at a site. Nevertheless, we strongly encourage additional sampling of polymorphic species and those whose taxonomy is poorly understood, especially when a taxon is known (or suspected) to include several taxa; for such species, samples of up to 25 males and 25 females may be adequate. Unless a species is unusually habitat restricted or stressed by disease or other factors, a sample of 25 specimens per class will not harm a population of small- to medium-size reptiles. Researchers interested in assessing genetic diversity within and among sites should remove tissue samples from 10 to 20 males and 10 to 20 females from each site for biochemical analysis and then preserve the specimens as scientific vouchers. For a more detailed discussion of specimen numbers for biochemical studies, see "Sample Size" under," Collecting and Preserving Tissues for Biochemical Analyses," in Chapter 7.

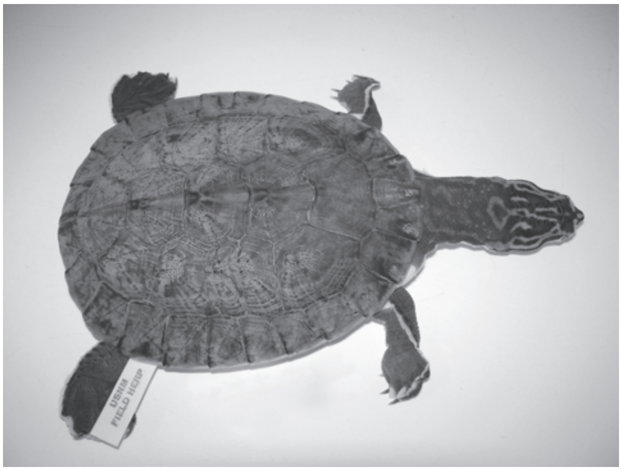

FIGURE 27 Voucher specimen of Geoffroy's Side-Necked Turtle (*Phrynops geoffroanus tuberosus*) from Kato, Guyana, cataloged at the U.S National Museum of Natural History. This specimen documents the first record of this taxon from Guyana. (Photo by R. P. Reynolds, U.S. Geological Survey.)

Factors other than sample size can also affect the potential for accurate identification of specimens. Improperly or carelessly prepared specimens are often difficult or sometimes impossible to identify because diagnostic features are obscured or modified. For example, hemipenis structure (Fig. 25) varies enormously and is an important taxonomic character, yet collectors often fail to evert a hemipenis (see Box 5, "Hemipenis Preparation," in Chapter 7) when preserving male lizards and snakes. Anyone collecting material for scientific purposes should be intimately familiar with proper techniques for preparing and documenting specimens (Chapter 7, "Preparing Voucher Specimens"). Photographs of representative specimens and notes on color in life (dorsal and ventral coloration, sexually dimorphic color patterns, iris color, and patagial, dewlap, and other display characters of lizards) often aid identification, as does ecological information. Both should be recorded in field notes. Generally speaking, a small number of carefully prepared specimens accompanied by detailed field notes is preferable to a large sample of carelessly prepared animals with minimal biological information.

Specimen Data

To fulfill their function as vouchers of inventory or monitoring studies, all specimens must be thoroughly documented. Data accompanying voucher specimens enhance their value and can facilitate identifications.

In addition to full locality data in a standard format and information on sampling procedures and habitat, the following documentation should accompany each voucher specimen (covered in greater detail in "Preparing Scientific Specimens," in Chapter 7, along with recommendations of other desirable data):

1. *Unique sample designation.* A unique field number is assigned by the collector to a specimen obtained at one place and time during a study. The number is printed or written in indelible ink on a field tag that is tied to the specimen with a square knot. Multiple preparations and/or secondary samples taken from a single specimen (e.g., skin, skeleton, tissue, parasites) are all assigned the same unique number.
2. *Date and time of collection.* The date and time (24-h clock) when the specimen was collected and the date it was prepared (if different) are essential. The month is written out, and the year is designated with a 4-digit number. Numeric designations and abbreviations are not used, because they are easily confused and often interpreted differently in different countries (e.g., 02/10/08 could be interpreted as February 10, 2008 or 2 October 1908).
3. *Collector.* The name of the collector (or collectors) is important, especially for referencing field notes. The collector's name is never abbreviated, and the middle initial is included if available.
4. *Field identification.* Ideally each specimen will eventually be identified to species. Because specific identification is often impossible in the field, however, a taxon name (e.g., family) or other distinguishing name (e.g., scincid, *Uta,* green snake) can be substituted for the scientific name until a definitive identification is made.
5. *Skeletal preparations.* The sexes of specimens roughed out in the field for preparation as skeletons are determined and recorded in the field catalogue. In addition, snout-vent (SVL) and tail (TL) lengths are measured and recorded.
6. *Other information.* The existence of special preparations (e.g., tissue samples, gut contents, parasites, photographs) and other specimen data (e.g., behavioral observations, color notes) are recorded in the field notes. Special preparations are referenced with the unique field number of the voucher specimen. Maps of the study area and trip itineraries are always useful for identification, cataloguing, and historical or archival purposes.

Most institutions require that the original collector's field notes and catalogue (or clear photocopies thereof) accompany any incoming collection. The importance of good field notes to efficient processing and all subsequent uses of a collection cannot be overemphasized. Incorrect or incomplete field data can seriously mislead a specialist and reduce the usefulness of specimens. If the data accompanying a collection are a secondary compilation from the original field notes, that should be clearly noted.

Specimen Repositories

Voucher specimens and their associated data should be transferred to a permanent repository as soon as practical after collection in order to prevent (or at least minimize) their deterioration in the field or in inadequate temporary storage facilities. Repositories must meet accepted standards of collection care, maintenance, and management (Karns 1986; Simmons 2002), and the specimens must be available for use by researchers and maintained and protected for use by future generations. All publications reporting results of inventory and monitoring studies should provide the name(s) and location(s) of the repository(ies) that houses the voucher specimens and accessory materials. Names of research institutions with large collections of reptile specimens are provided in Appendix I.

The amounts of time, space, and money required to maintain a museum collection are enormous, and relatively few institutions are able to provide the long-term security necessary for large research collections. Therefore, selection of an appropriate institution for the deposition of scientific voucher specimens is of critical importance and must be guided by the welfare of the collection. Using collections as a means of obtaining employment, acceptance to graduate school, or other favors is inappropriate. Maintaining a private collection unavailable for study by other qualified researchers does a disservice to the scientific community and may imperil the long-term survival of the specimens. Important collections have been lost or destroyed when the collector died or retired and his/her home institution lost interest in the collection or lacked the resources required for its maintenance and did not realize its importance.

When a researcher from one country collects specimens in another country, it is highly appropriate, and often a requirement of the collecting permit, for representative material or some percentage of the collection to be deposited there in a designated institution. This can be done on return from the field, or the material can be sent to the institution after identification. Such material should become part of a functional reference collection documenting the biodiversity of that country. Excessive nationalism or misplaced possessiveness, however, should not obscure the economic realities of establishing and maintaining a functioning research facility for extensive natural history collections. The primary concerns of all responsible biologists and local officials should be the long-term maintenance of specimens and associated data and their availability to qualified scientists for study.

Several variables (see discussion in Lee et al. 1982) influence the choice of a collection repository. If identifications are required, an institution that has a history of research in the geographic area, an appropriate specialist on the staff, and access to an extensive library is optimal. The prospective donor should, however, obtain a statement of the museum's policies regarding acquisition, preservation, maintenance, and *deaccessioning* (formal process for permanently removing a specimen from a museum collection, with an appropriate transfer of title) of collections to determine if they meet his or her needs. Most institutions will honor reasonable requests from a donor, but policy is determined by many other factors as well.

The identification, distribution, and cataloguing of voucher collections is a service provided by most natural history museums to the scientific community. Unfortunately, many museums are currently plagued by limited budgets and staff shortages. Identification and incorporation of voucher specimens into a large collection are time-consuming activities. A staff member may spend hours searching the literature for appropriate information. In addition, she or he may have to visit another institution or borrow specimens to compare incoming material with existing collections, or send the new material to specialists for identification. Altruism, if it exists, has its limits. Few museums can afford to invest the time and energy required to verify identifications of specimens in a major collection without acquiring at least a portion of the collection. For example, an investigator who asks staff at an institution to assist with specimen identifications but intends to deposit the collection elsewhere should at least offer to deposit representative materials in the institution that provides the identification service. Some donors (e.g., U.S. National Park Service) expect institutions to maintain their voucher collections as discrete units, separate from the main collections. Realistically, however, space and curatorial support at most institutions is limited, making accommodation of such requests unlikely. Whether a voucher collection should be maintained in a single institution or distributed among several is also debated. Each option has merit. The first obviously simplifies future study of the collection; the latter provides for greater access by researchers in different geographic areas. A donor should review an institution's exchange policy before depositing his/her specimens there.

Conclusions

Voucher specimens taken during faunal surveys and their accompanying field notes and associated data have almost incalculable scientific value and are an essential part of the scientific method. Given the inevitable widespread habitat destruction that may preclude collection of additional material from already-sampled areas or similar habitats, for example, these specimens become critically important for documenting global biodiversity. Likewise, the rapid technological advances currently being made likely will lead to new, previously unsuspected uses of these specimens and their data. We should view this irreplaceable "time capsule" of information as part of our scientific heritage and work to ensure that it is permanently stored at institutions with documented long-term commitments to conserving specimens and making them available for study by qualified researchers.

CHAPTER SEVEN

Preparing Reptiles as Voucher Specimens

MERCEDES S. FOSTER
Chapter Coordinator

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Overview

Mercedes S. Foster and R. W. McDiarmid

When early explorers began charting the vast reaches of the Atlantic, Pacific, and Indian oceans, they discovered and visited many seemingly exotic places generally unknown to Europeans. Basic trade routes to the Orient were known, but the extensive and complex insular environments of the Indo-Australian region remained *terrae incognitae,* as did the interior regions of Africa and the New World, where they eventually encountered thriving, sophisticated civilizations. During explorations of and visits to these areas, the crews of the sailing ships often acquired unusual and colorful cultural and natural history objects, which they carried back to their home ports as mementos of their travels and records of their discoveries. Some of these items, including seeds, bones, shells, and other plant and animal parts, found their way into the private collections and "cabinets of curiosities" that were fashionable in the late 16th and early 17th centuries among many wealthy Europeans. Nevertheless, in the absence of any systematic effort to understand and catalogue nature, the articles remained simply novelties.

During the early stages of scientific development in 18th- and 19th-century Europe, there was a general awakening of interest in the order and diversity in nature. The Swedish naturalist Carl Linnaeus began compiling lists of species and arranging organisms into natural categories. In the 10th edition of his *Systema Naturae* (Linnaeus 1758), he introduced a system for naming and cataloguing animal diversity with a two-part name (*binomen*); his method continues to be used today. Scientific societies were established to identify species and assemble lists, and institutions to house and maintain specimens were founded (Asma 2001). Not surprisingly, the return of a ship from an exploring expedition engendered much interest among the educated populace, and collections of natural history objects that included new and generally unfamiliar species attracted considerable attention. Documenting the natural diversity of the world became a goal, and collecting (and illustrating) species from unknown areas gradually became an important element of the exploring effort, rather than merely a source of curiosities for private collections. So great was the interest that some naturalists were able to make a living collecting specimens for museums, particularly of new species from unknown areas. In fact, many ships of the 18th and 19th centuries took along naturalists whose sole responsibility was to catalogue and collect examples of new life forms they might encounter.

Many of these early vouchers looked similar to those we see today, but rarely was any particular species represented by more than one or a few specimens, and the information that accompanied them, if any, was at best rudimentary by today's standards. The skins of most mammals and birds were removed from the body and simply dried or prepared as taxidermy mounts; the carcasses were usually eaten or discarded. Others animals such as reptiles, amphibians, and invertebrates were generally preserved whole in alcohol (often rum or other drinkable substance) or dried; many were known only from hard parts (e.g., turtle or mollusk shells, mammal skulls). The primary function of these vouchers was to document and illustrate the kinds of organisms occupying these unknown lands, initially in order to satisfy the curiosity of those unable to visit them, and to identify species that might provide a marketable product (e.g., fur, food, spice, or fiber). Later they became part of the effort to document the natural diversity of the planet. Topics whose study requires large samples from wide

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geographic areas had not yet been conceived, and the value of associated data (information on sex, age, reproductive condition, diet, and so forth) to future scientific study had not yet been recognized. Nevertheless, Linnaeus's nomenclatural system and attempts to group like kinds of organisms into genera and families presaged taxonomy and systematics, and eventually scientists realized that many investigations would benefit from and eventually rely on many types of accompanying data. In addition to being unaware of fields of study that had not yet been invented, early explorers and scientists were constrained by a lack of tools and equipment that today we take for granted. Pesola scales were centuries in the future, as were Dewar flasks and topographic maps, let alone Global Positioning System (GPS) devices. Locality data more detailed than "Nouvelle-Hollande," "Indiis," "flumen Amazonum," or "America" were generally lacking, because explorers often did not know where they were and were just starting to assign European names or adopt local names to those places.

In the past 50 to 75 years, the fields of biological inquiry in which voucher specimens of plants and animals play a central role have expanded beyond anything that could have been anticipated. Dry skins and fluid-preserved specimens are used not only to document species occurrences but also as a basis for systematic, biogeographic, evolutionary, ecological, and other studies. Tissues have become the basis for genetic studies, and fluid-preserved specimens and skeletons are critical for anatomical investigations. Many studies of endo- and ectoparasites have originated with specimens of host organisms preserved in museum collections, and epidemiologists rely heavily on those same specimens and their associated data to determine likely distributions and paths of the spread of some diseases. Likewise, developmental series of organisms have provided considerable insight into the evolution of specific morphological traits. Well-prepared voucher specimens have been and will continue to be essential to many aspects of scientific enquiry. With the tools now available, we can preserve different parts of an organism in different ways so as to support multiple types of analyses. We also know enough about the significance of different characteristics (e.g., particular scales, hemipenes, toes) in studies of phylogeny or functional morphology to preserve specimens in ways that make the traits most easily measured, X-rayed, or otherwise analyzed. The scientific importance of well-prepared and well-documented vouchers cannot be overstated (see Chapter 6, "Voucher Specimins"), although regrettably, it remains underappreciated.

In the sections below, the authors discuss the ways in which different types of specimens should be preserved and positioned to ensure their indefinite persistence and their maximum utility. They review types of data to be recorded and standards for recording them. Assuredly, new methods of preservation will arise, and new characters of importance to various types of studies will be recognized as will new ways to analyze known characters. Chapters like this one will be revised and extended in future publications as our knowledge expands. For now, however, we urge collectors to embrace the protocols detailed here.

One other point deserves mention. In past centuries when explorers traveled the world discovering unknown organisms and preserving specimens, species generally were not yet threatened by anthropogenic factors (but see Olsen and James 1982; Steadman et al. 1999; Duncan et al. 2002), or at least we were unaware of such threats. No treaties, conventions, or laws enforced by government permit agencies restricted or regulated the collection of organisms or their trade for whatever purpose, and organizations whose members would ban the use of animals for medical and other research, for teaching (e.g., dissections), and recreation (e.g., hunting and fishing) had yet to appear. Today, all of these factors greatly influence the ability of scientists to collect animal specimens, even those presently unknown to science. Yet new species continue to be discovered at a prodigious rate, even among vertebrates, and especially among reptiles (see Uetz 2010, 2011), as new areas are explored and old areas revisited. Consequently, the value of every specimen obtained continues to increase, and the need to maximize the quality and utility of each one has never been greater.

Preparing Scientific Specimens

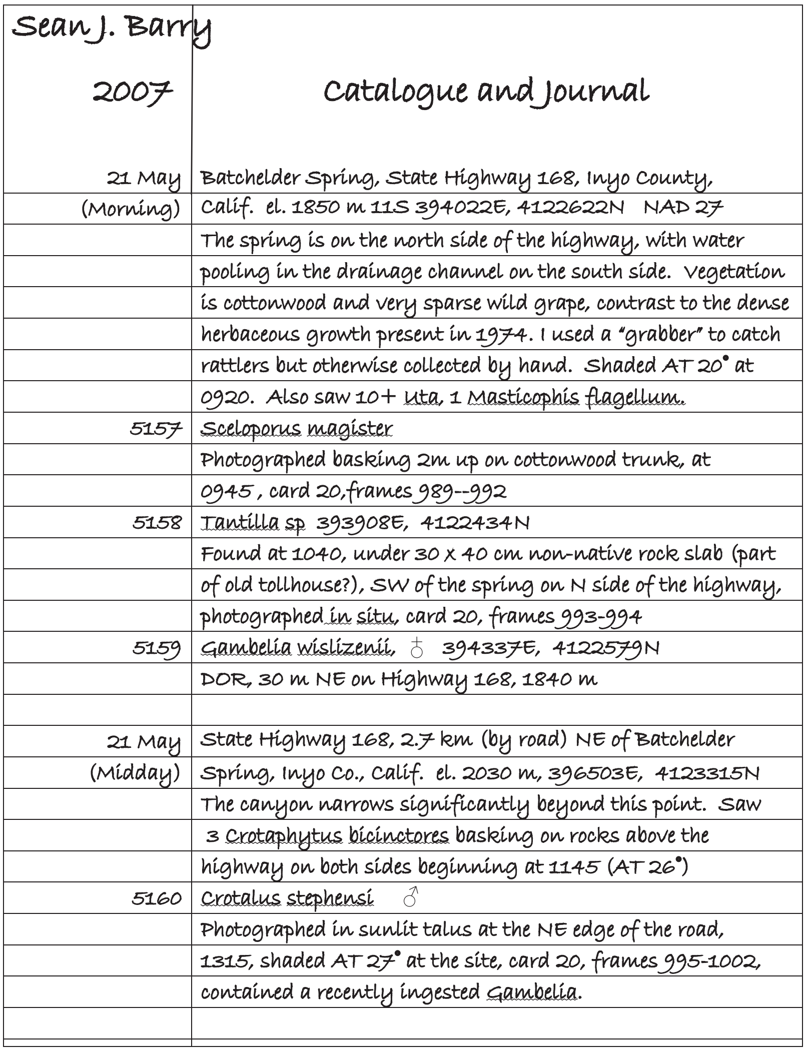
Sean J. Barry

Museum voucher specimens are important products of biological surveys. Photographs and field notes may supplement vouchers, but for definitive identification there is no credible substitute for an accurately documented and tagged, permanently preserved specimen. Voucher specimens and specimen series are also important for systematics, and ecological and evolutionary studies.

The goals when preparing scientific specimens of reptiles are to preserve them and their associated documentation permanently and to maximize their utility for future studies. The quality, utility, and longevity of voucher specimens depend almost entirely on how well they are prepared and documented in the field, and subsequently, how well they are maintained in a museum collection. Techniques for specimen preparation have evolved considerably over the past century as our understanding of fixation and the effects of storage chemicals on animal tissue has increased and the equipment and supplies used to process specimens have become more serviceable and portable (e.g., pocket electronic balances) and function under a broader range of rugged conditions. At the same time, advancements in technology have led to devices that allow investigators to obtain more precise and reliable data (e.g., GPS units that record locations with sub-meter accuracy, digital cameras and macrophotography that allow for better color records, portable Dewar flasks for ultra-cold storage of tissue samples). In addition, investigators have developed an appreciation of the value of associated specimens (e.g., tissue samples, eggs, parasites, photographs) and expanded data (e.g., notes on behavior, microhabitats, etc.) and now routinely collect materials and record information that significantly increase the breadth of studies to which specimens may contribute.

In this section, I outline the stages of specimen processing and permanent preservation from field documentation through euthanasia, fixation, preservation, tagging, preparation of oversize specimens, field storage, and packing and shipping of specimens from field locations. C. J. Raxworthy has also provided special instructions for the preservation of hemipenes (see Box 5, "Hemipenis Preparation," below). I recommend certain practices and techniques that are known to produce high-quality herpetological specimens, but the best results will come only with practice and experience, careful work, and attention to detail. Some useful references on the topics in this chapter include Anderson (1965), Pisani (1973), McDiarmid (1994, for amphibian vouchers), and Simmons (2002).

Several of the techniques I describe involve the use of hazardous chemicals. Investigators should familiarize themselves



Sean J. Barry	
2007	Catalogue and Journal
21 May	Batchelder Spring, State Highway 168, Inyo County,
(Morning)	Calif. el. 1850 m 11S 394022E, 4122622N NAD 27
	The spring is on the north side of the highway, with water
	pooling in the drainage channel on the south side. Vegetation
	is cottonwood and very sparse wild grape, contrast to the dense
	herbaceous growth present in 1974. I used a "grabber" to catch
	rattlers but otherwise collected by hand. Shaded AT 20° at
	0920. Also saw 10+ *Uta*, 1 *Masticophis flagellum.*
5157	*Sceloporus magister*
	Photographed basking 2m up on cottonwood trunk, at
	0945 , card 20,frames 989--992
5158	*Tantilla sp* 393908E, 4122434N
	Found at 1040, under 30 x 40 cm non-native rock slab (part
	of old tollhouse?), SW of the spring on N side of the highway,
	photographed *in situ*, card 20, frames 993-994
5159	*Gambelia wislizenii*, ♁ 394337E, 4122579N
	DOR, 30 m NE on Highway 168, 1840 m
21 May	State Highway 168, 2.7 km (by road) NE of Batchelder
(Midday)	Spring, Inyo Co., Calif. el. 2030 m, 396503E, 4123315N
	The canyon narrows significantly beyond this point. Saw
	3 *Crotaphytus bicinctores* basking on rocks above the
	highway on both sides beginning at 1145 (AT 26°)
5160	*Crotalus stephensi* ♂
	Photographed in sunlit talus at the NE edge of the road,
	1315, shaded AT 27° at the site, card 20, frames 995-1002,
	contained a recently ingested *Gambelia*.

FIGURE 28 Typical page from a field catalogue.

with the hazards associated with handling these chemicals as well as with the safe practices to be followed when using them. Useful references include the most recent edition of Sax's Dangerous Properties of Industrial Materials (Lewis 2004) and current Material Safety Data Sheets (MSDS) available from the manufacturers of each chemical. Investigators should also stay current on relevant regulations for transport, storage, use, and disposal of hazardous materials (see "Permits and Regulations," in Appendix II). Most important, every investigator must ensure that everyone in his or her group is properly trained and equipped to handle these materials safely.

Field Notes

Investigators should maintain a field book in which they record detailed observations and collection notes, which are nearly as important as the specimens that they accompany. Typically, the field notes include a journal and a specimen catalogue that are organized chronologically, following the itinerary of the collecting trip. For each locality visited, the collector describes in the journal the site and the habitat(s) present, the weather and physiography, the sampling methods used at the locality, and any information that may assist individuals consulting the notes in the future (e.g., names of colleagues present, local contacts, local sources of hard-to-find supplies, such as liquid nitrogen). The collector should include a general map of the area indicating roads, topographic features (streams, lakes, rocky outcrops, etc.), and vegetation (e.g., pine forest, chaparral, clearing). Such maps are extremely useful to future workers learning to navigate the area or interested in changes to the area that have occurred between visits.

The specimen catalogue consists of daily entries that individually list all specimens collected at each site on each date (Fig. 28). Specimens are entered in loose time groups (e.g., collected in the morning vs. at night) and assigned numbers sequentially. Each fieldworker should catalogue, number, and tag (see "Specimen Tags," below) all specimens that he or she collects. As discussed in detail below, collectors typically use field tags preprinted with sequential numbers preceded by the collector's initials. The number sequence generally continues throughout a collector's lifetime. On any given field trip, numbering begins with the first sequential number following the last number used on the most recent previous trip. Collectors should avoid any personal numbering system that duplicates numbers, such as systems that reset annually (e.g., 98-0001, 99-0001) or that incorporate the date (e.g., 10132010-001 or 13102010-001).

Catalogued specimens are identified to whatever taxonomic level is known along with an identifying description (e.g., "yellow lizard," "flat-nosed boid," *Crotalus* sp., *Dermochelys coriacea*). Habitat and specimen photographs and associated items (e.g., a shed skin) collected during field excursions should also be catalogued. All items associated with a particular specimen, such as photographs of the specimen, stomach contents, eggs, tissue samples, and parasites, carry the field number of that specimen and should also be catalogued under that number. Many workers also record metric data such as head-body and tail lengths, specimen weight, and color descriptions of any body part that may fade in preservative. A description of the microhabitat (see "Describing Microhabitats," in Chapter 4), the animal's behavior, and any other relevant observations or data not already recorded in the journal account are also included (Fig. 28).

To help simplify the field book, some collectors combine the journal and catalogue into a single narrative, particularly on short trips or when few specimens are collected. Conversely, field biologists who conduct detailed natural history studies and also collect and preserve specimens may add individual multipage species accounts to the field book (the "Grinnell System," named for Joseph Grinnell). Such information is potentially useful for natural history studies focusing on only one or a few species, and species accounts provide a robust way to record information on species that were observed but neither collected nor photographed during a field excursion. However, the species account system is otherwise cumbersome and time consuming, and most workers maintain only a journal and specimen catalogue. Collectors who use the journal/specimen catalogue format without species accounts should take care to record observations of species observed but not collected or photographed in the daily journal (not in the specimen catalogue).

I recommend that investigators record data on loose-leaf pages carried in a small three-ring binder. Catalogue and journal pages can be grouped and numbered independently, or they can be integrated into a single chronological record. The month, year, and collector's name should be noted at the top of each page. New pages are added wherever necessary, and all pages used can be removed after each trip is completed. The collector should carefully safeguard loose-leaf pages to prevent loss during field excursions or on return to the laboratory. Some investigators photocopy their field notes and specimen catalogue as soon as they return from the field and store the duplicates at a different location from the originals.

The best choice for field book and catalogue pages is 100 percent rag paper (e.g., Resistall), ruled only on one side, and with a 2.5- to 3.75-cm (1–1.5-in) left margin. Page size can vary from 15 × 22 cm (6 × 9 in) to 21 × 28 cm (8.5 × 11 in). If Resistall paper is unavailable, any acid-free white rag or linen paper is suitable as long as permanent ink or pencil remains legible after being rubbed vigorously or wetted. A pencil, a pen with permanent black ink, such as Higgins Eternal (preferably), or any of several newer permanent-ink pens, such as the Pilot permanent ultra-fine point marker (catalogue no. SCA-UF) or the Sanford Uni-Ball, should be used when writing field notes or cataloguing specimens.

Some investigators prefer to use durable, hard-bound notebooks or stapled notebooks such as the 11.7- × 17.8-cm (4⅝- × 7-in) yellow "Rite in the Rain" all-weather notebooks (J. L. Darling Corp. and Forestry Suppliers). J. L. Darling Corporation recommends that pencil or their proprietary permanent pen be used in their Rite in the Rain books, but some other choices such as the Pilot mentioned above are also suitable. Investigators that use this type of notebook must initiate a new book with each field trip; a field book with original data should never be taken back into the field.

Other commonly used data-recording systems include loose-leaf, preformatted data sheets and electronic (digital) data entry devices. The "fill-in-the-blank" data-sheet format is convenient and uniform and generally ensures that categorical data will always be recorded. However, it is also bulky and inflexible—depending on format and layout, an entire two-sided data sheet may replace a quarter-page journal and catalogue entry and yet may not accommodate extensive notes. Electronic data recording devices, such as personal digital assistants (PDAs) and notebook computers are convenient (see "Handheld Computers in Digital Data Collection," in Chapter 4), but they are vulnerable to damage or spontaneous failure and consequent data loss, especially under harsh field conditions. Investigators who use electronic devices should back up their data frequently on diverse media (optical disks, portable hard drives, and "thumb drives"), print hard copies of the data whenever possible, or supplement digital data entry with a handwritten specimen catalogue.

Processing Specimens

LIVE SPECIMENS

Prior to processing, most live, nonvenomous reptiles can be held and transported in cloth bags (socks, pillowcases, custom-made bags of various sizes) or other secure, compact containers, unless external parasites are to be collected (see "Collecting and Preserving Parasites During Reptile Biodiversity Surveys," later in this chapter, for alternative holding methods). Ziplock and other plastic bags of various sizes are also convenient and widely used, but they are potentially inhumane unless pierced in several places to allow air circulation. Collectors should insert crumpled paper or leaves into the bags to hold them open and give the animals space in which to move. Bag size should be appropriate for the size of the animal, with one specimen per bag. Most herpetologists favor heavy unbleached muslin bags with double-hemmed edges and seams (to prevent specimens from becoming entangled in unraveling threads) for nonvenomous species. Bags should be checked frequently for holes and weak spots. A knot (not a drawstring) is the most secure way to close a cloth bag.

Venomous reptiles should be held and transported in secure, hard-walled containers, such as tight wooden boxes with lids that have gaskets, or in plastic containers (e.g., Tupperware, Rubbermaid) with ventilation holes and positive sealing or locking lids (perhaps reinforced with duct tape). However, this can be impractical when collecting away from camp, so most collectors transport venomous snakes in cloth bags, transferring them to hard-walled containers back at camp. Although convenient, this practice can be dangerous; many venomous snakes are capable of biting through a cloth bag, and investigators must take care to transport them safely. Venomous snakes should be double-bagged and the bag carried at the end of a stick, away from the collector's body (i.e., not looped in the collector's belt). Fingers are also vulnerable when a plastic container harboring a venomous snake is opened, so it should be tipped to keep the animal away from the opening. Any container holding a venomous animal must be prominently labeled. Very large reptiles require special treatment to ensure safe handling and transport. Fowler (1995) has discussed techniques for the humane capture and restraint of large, dangerous reptiles (see also "Handling Live Reptiles," in Chapter 8).

As soon as a researcher collects a specimen, she or he should immediately record it in a small pocket notebook (not the field book discussed above) with a consecutive "daily number" and pertinent locality and habitat data, time of day, and behavioral or other observations. The daily number is also noted on the container. Plastic containers and cloth bags can be numbered permanently with a Sharpie or with a tag attached to the bag. The drawback of permanently numbered specimen bags and containers is that the collector must sort through the containers to find the desired number for each specimen to maintain consecutive numbering. Alternatively, the specimen number can be written with a Sharpie pen on the container when the specimen is collected, or on a plastic tag that is placed in the container or bag with the specimen. Sharpie inks can be removed easily from the surfaces of hard plastic containers or plastic bags with 70 percent ethanol. The bag or container number is part of the temporary daily log. The collector's field number replaces the container number when the specimen is processed.

EUTHANASIA

Most workers use the barbiturate drug sodium pentobarbital (Nembutal [not Nembutal syrup elixir], Beuthanasia, and generic preparations, 100–400 mg/ml aqueous solution) to kill reptiles destined for scientific collections. An intracardiac or intracoelomic pentobarbital injection (100 mg/kg body weight) induces profound central nervous system compromise and rapid, seemingly painless death in reptiles. In practice, 0.1 to 0.25 ml of a 100-mg/ml solution is appropriate for a *Sceloporus*-size lizard, 0.25 to 0.5 ml for a 15-cm turtle or 50-cm snake, 1.0 ml for a snake up to about 1,200 mm (4 ft) total length, and up to 5 ml or more for larger specimens. The dose should be increased if death is not extremely rapid. Respiration and heartbeat usually stop immediately, and the euthanized specimen remains relaxed. Some investigators dilute sodium pentobarbital to only 5 to 40 mg/ml in water and inject somewhat larger volumes than those provided above, because it is easier to inject sufficient material when the dose volume is so much greater. However, reptiles sometimes take longer to expire when dosed with a dilute solution, and investigators should be careful to avoid overinjection and bloating. Cardiac puncture is relatively easy in large squamates and crocodilians because the heart can be palpated or its location visualized fairly easily. The needle is inserted ventrally just posterior or lateral to the heart and advanced until slight resistance is encountered. The syringe is aspirated slightly—blood in the hub indicates that the needle is properly sited within the ventricular lumen. Intracoelomic injection is more practical for small squamates, turtles, and venomous species. The needle is inserted ventrally or ventrolaterally into the coelomic cavity, and the drug is injected. If there is substantial resistance to the plunger, the needle should be repositioned until the solution flows out smoothly. Detrimental effects of sodium pentobarbital on specimen processing or storage or on downstream applications such as molecular or histological work have not been reported. Sodium pentobarbital solutions that are discolored or contain precipitates should be discarded.

One person can usually restrain a small- to medium-size nonvenomous reptile in one hand and inject with the other, but one person (or more) may be required to restrain a larger animal while another person injects the drug. Dangerously venomous species obviously require special handling. One way to restrain a venomous snake is to induce it to crawl into an acrylic plastic (Plexiglas) tube (Murphy and Armstrong 1978; Fowler 1995). The tube is capped at one end and sized so that the specimen cannot turn around once inside. Machined slots or holes in the tube wall allow access for air as well as injection. Most viperids and some elapids can also be restrained with wire mesh. One person holds an oversize piece of strong mesh tightly against the animal so that it is pressed against a flat surface, and another person injects the drug intracoelomically through the mesh. A special wooden "squeeze box" with a screened, movable lid can be used to immobilize large viperids and large active elapids for drug injection (see Fowler 1995 for building instructions). Some very experienced (and foolish) workers pin venomous snakes with a "snake hook," grasp the snake behind the jaw, and restrain it tightly while another person injects the drug. Handling venomous species is obviously hazardous and requires extensive training; investigators should practice any of the above restraint techniques first with nonvenomous species. Further techniques for handling venomous species are discussed in detail later in this volume (see "Handling Live Reptiles," in Chapter 8).

The sole but very important drawback of sodium pentobarbital is that it is a controlled substance subject to tight regulation in most countries. In the United States the drug is covered by federal Drug Enforcement Agency (DEA) Class II rules, and investigators need appropriate state and federal permits to acquire and use it. They must also follow strict rules of record keeping, custody, and storage. Careless management of controlled substances can result in severe legal penalties. The DEA also regulates sodium pentobarbital export, and similar agencies in other nations regulate its import, often under complex laws. Investigators planning research in any country should arrange in advance for required permits to import or purchase, possess, and use the material legally.

Some workers euthanize reptiles with an intracoelomic injection of a saturated aqueous solution of chloretone (hydrous chlorobutanol), a standard amphibian euthanizing agent (McDiarmid 1994). An injection of 0.5 to 1.0 ml seems sufficient to kill specimens of less than 50-g body mass; larger specimens require more. The anesthetic effect is rapid, but death may take up to 30 minutes. Livezey (1958) euthanized reptiles effectively by intracardiac injection of the local anesthetic procaine hydrochloride (lidocaine, 500 mg/kg body mass). Chloretone and procaine are free of the complex regulatory web that surrounds barbiturates, so investigators should consider using them where regulations for other drugs pose difficulties. Chloretone is available from chemical suppliers; individuals wishing to purchase lidocaine usually need a prescription from a physician or veterinarian.

Some workers have used inhaled anesthetics, such as ethyl ether and chloroform, to euthanize reptiles (Anderson 1965). A cotton ball saturated with the volatile chemical is placed with the specimen in an airtight metal or glass container. Euthanasia is said to be rapid, although the specimen remains stiff and contorted for some time after death (Stebbins 1954). Despite that drawback, inhalant euthanasia may be the best way to deal with venomous snakes in the absence of special equipment, or at least to immobilize them for safe handling and subsequent pentobarbital injection. Because many inhalants are flammable or highly toxic, they present special transportation, handling, and storage problems. For example, ethyl ether is very flammable, and it also combines with oxygen to form unstable peroxides as it ages, so an old opened metal can of ethyl ether with the typical plastic pry-cap is a potential explosion hazard (Kelly 1996). Chloroform is toxic and a suspected carcinogen (Lewis 2004), and like ether, it is highly volatile. These dangerous chemicals should be used outdoors or in a chemical fume hood. Some older references recommend freezing, immersion in warm water, exposure of confined animals to direct sunlight, and pericardial or intracardial injection of formalin to euthanize reptiles. I do not recommend any of these, because they are likely inhumane, may cause processing problems that result in lower-quality specimens, or may lead to long-term storage problems.

Specimens should be preserved within a few hours of being euthanized, especially in very warm weather. Dead specimens can be refrigerated at 4°C (39°F) for up to 1 day, but immediate fixation is preferable. If possible, specimens should not be frozen prior to fixation because freezing causes post-fixation problems such as excessive softness, curled toes, and epidermal exfoliation (Scott and Aquino-Shuster 1989). Specimens that smell bad, that are stained green ventrally (from bile leakage), or whose sides have "caved in" are putrefied and may be unsalvageable. Road kills can be important voucher specimens, but many animals found dead on the road (DOR) are so badly damaged that preservation may not be worthwhile. The importance of the specimen should dictate how it is handled. For example, if a DOR specimen is the sole representative of its species in a biodiversity survey, the investigator should try to preserve some taxonomically identifiable part. Such specimens can sometimes be eviscerated and then prepared as osteological specimens.

PHOTOGRAPHIC RECORDS

Many investigators routinely photograph reptiles before or immediately after euthanasia to document colors that may change or fade in preservative. A tripod-mounted 35-mm single-lens-reflex (SLR) film camera or equivalent SLR digital camera with a high-quality lens (e.g., a true 50–180-mm macro lens) is the most practical type for specimen documentation in the field. The photographer positions the specimen in a lifelike pose on the ground, places it in a suitable habitat, or sets it on a uniform neutral background (with supplemental lighting, if needed) and photographs the dorsal, ventral, and lateral surfaces and close-up details of scale or scute arrangements. The photographs are useful for identifications. Collectors should include a label with at least the field-catalogue specimen number in the photograph. The film roll or memory stick and frame numbers of all photographs should also be recorded under the specimen catalogue entry in the field book. Some slide films reproduce specimen colors better than others, which tend toward excessive graininess or unnaturally saturated colors. Print films also tend toward unnatural hues and should never be used for specimen documentation. Color reproduction is also affected by lighting source, angle, and direction; film lot; exposure; and lens quality. The investigator should experiment with all of these factors to standardize a photographic strategy. Shaw (1987) has provided useful information on close-up ("macro") photographic techniques.

Digital SLR cameras eliminate many of the problems of film photography and introduce almost none of their own. An inexpensive "point and shoot" zoom 35-mm film camera or comparable digital model with an optical zoom lens is adequate for most habitat and collecting-site photography. Inexpensive waterproof digital models that can document underwater habitat are also readily available. However, most digital and automatic film SLR cameras are relatively delicate and subject to failure, so investigators should consider carrying a spare, manual-control film SLR in the field. Excellent older-model manual-control SLRs, along with compatible lenses, flash units, and other accessories, are readily available very inexpensively from used camera dealers. A separate light meter is a critical adjunct to any of these older SLR cameras.

FIXATIVES

Most workers use formaldehyde as the primary museum and histological fixative, because it is effective and inexpensive. Formaldehyde penetrates tissues quickly, arrests and prevents autolysis, hardens a specimen so that it can be handled and examined without damage, and preserves all morphological details except color. Formaldehyde is an acrid, colorless, toxic gas that is sold as a 37–40 percent unbuffered aqueous solution, typically with 10–15 percent methanol added as a stabilizer. One part of commercial formaldehyde is diluted in nine parts water to make "10 percent formalin," the standard tissue and whole-mount fixative. This is a major advantage for field excursions because outward-bound 37 percent formaldehyde occupies only 10 percent of its final working volume, most of which will be incorporated into specimens or discarded for the return trip.

Formalin has numerous photochemical effects, but it hardens soft tissues and stabilizes macromolecules primarily by covalently cross-linking peptide end groups with aldehyde groups. Aldehyde linkage is most efficient and thorough at very low pH and high temperature, obviously not suitable for museum specimen preparation. Unstable aldehyde linkages formed at neutral pH and ambient temperatures may "dissolve" in water (Pearse 1980), so post-fix rinses with water should be curtailed or avoided, although they may be required if the specimens are to be transported by air. Formalin is slightly acidic, and it shifts pH several times during the fixation process, which tends to decalcify bone and clear soft tissues (Taylor 1977; Pearse 1980). Consequently, most workers buffer and neutralize formalin with mono- and dibasic sodium phosphate (NaH_2PO_4 and Na_2HPO_4, respectively). Investigators carry small plastic bags or vials on field excursions prepackaged separately with 4.0 g of monobasic and 6.5 g of anhydrous dibasic sodium phosphate, which is enough to treat 1 liter of 10 percent formalin. In the laboratory, formaldehyde is diluted to working strength with distilled or deionized water, but in the field the cleanest available local water may be rain water. The pH of buffered 10 percent formalin should be checked with indicator paper or with an inexpensive handheld pH meter, and if necessary adjusted to pH 7.0 to 7.2 with 5M hydrochloric acid or 5M sodium hydroxide (if available). The acidity of mineral-free water diluents is quickly neutralized in buffered formalin. Some workers use calcium or magnesium carbonate in the form of marble chips or powder instead of sodium phosphate to neutralize formalin, and others have recommended sodium borate (borax; e.g., Pisani 1973). However, borate-buffered formalin tends to clear fixed tissue and should be avoided (Taylor 1977).

Formalin is extremely unpleasant and hazardous to handle. It is flammable, toxic, and irritating, and it is an experimentally confirmed carcinogen (Lewis 2004). Most occupational safety guidelines specify that it be used only outdoors or in a chemical fume hood because it is easily absorbed across the lungs and skin. Complying with these rules in the field is usually easy. Formalin's "tanning" effect on bare skin has long annoyed herpetologists, and some workers have developed a contact dermatitis or severe systemic allergy from repeated exposure to formalin. Thin latex or nitrile clinic gloves offer little protection against such a harsh chemical. Heavy neoprene gloves are much better, and I recommend that all workers, sensitized or not, consider using neoprene gloves to handle formalin or formalin-fixed material.

It is also illegal to ship formalin by routine common carrier except under special conditions—a container failure could cause an aircraft emergency. Formalin must be packaged and labeled to U.S. Department of Transportation (DOT) and International Air Transport Association (IATA) standards and shipped by carriers equipped to handle hazardous materials. Most of the fluid chemicals discussed in this chapter (e.g., ethanol, chloroform, and ether) must be handled the same way. Packaging standards are updated periodically, so investigators should obtain current packaging instructions from the carrier or from specialists in hazardous materials shipping. Air transport of specimens preserved in formalin is also governed by ever-changing regulations. Investigators should always consult the most recent versions of DOT and IATA (e.g., IATA 2010) regulations.

Waste formalin must be discarded according to local regulations. Many institutions and municipalities require that formaldehyde be packaged and disposed of as hazardous waste, not in wastewater. Hazardous waste disposal is very expensive and often inconvenient, but all locations within the United States (and many in other countries) are likely to adopt these rules eventually. Workers and their institutions must comply with these regulations and use formaldehyde as efficiently as possible.

Ideally, 37 percent formaldehyde (= 100% formalin) should be obtained from reliable sources at home and shipped or carried to the field destination, to ensure its quality. This is easy within the United States, but many countries currently prohibit the import of hazardous materials, and in any case, the costs of shipping hazardous materials have skyrocketed. Thus, collectors often must purchase formalin locally, which can be problematic because quality may vary from country to country. For example, old formalin (which may be the only formalin available) often contains fairly high levels of formic acid (check the pH!) and may not fix tissues well (Pearse 1980). Fieldworkers should always monitor the fixation process carefully, being especially attentive when questionable formalin has been used.

Ethyl alcohol or ethanol (70% in distilled or deionized water) is also a good fixative. It hardens tissues less, but bleaches tissues more, than formalin and is a far less toxic, but more expensive, alternative. Ethanol solutions are typically pH neutral and do not require added buffer. The principal disadvantages of ethanol are that it is not always available or not available without a license, it is often heavily taxed, and it is a hazardous (flammable) material that must be shipped according to DOT and IATA specifications. In addition, DNA extracted from alcohol-fixed, old specimens may be too degraded for analysis. Disposal of alcohol is also subject to hazardous waste regulations. Isopropanol (50%) has also been used as a fixative, but it is far less satisfactory than ethanol. Ethanol is also the fixative of choice for specimens destined for preparation as cleared and stained bony and cartilaginous skeletal material (Hildebrand 1968; Simons and van Horn 1971), especially from small reptiles. Formalin-fixed specimens rarely "clear" completely in subsequent alkali maceration.

Special histological fixatives such as formalin-acetic acid-alcohol (FAA), glutaraldehyde, and Bouin's (PFA) solution can be used as primary whole-animal fixatives, although these solutions are more complicated, more expensive, and potentially even more hazardous (such as the explosive picric acid in Bouin's) than routine 10 percent formalin. Paraformaldehyde, or formaldehyde that has been polymerized under pressure to form a dry powder, is somewhat easier to transport than formaldehyde but is still considered to be a hazardous material. It is an ultra-compact source of specimen fixative (Taub 1962; Huheey 1963), but many workers consider it markedly inferior to formaldehyde as a whole-animal fixative (Saul 1981). Paraformaldehyde dissolves most efficiently with added heat and alkali, and the pH of the solution may require adjustment.

FIXATION

Reptiles are difficult to preserve because fixative does not penetrate the skin but must be injected with a hypodermic syringe and needle to perfuse the specimen thoroughly. To fix a reptile, the preparator injects 10 percent formalin into all body cavities and muscle masses with a 10- to 20-cc syringe with a Luer lock (threaded twist-on fitting at the tip of a syringe that keeps it securely connected to a needle or other device) and an 18-gauge or smaller needle. Safety goggles prevent formalin from splashing into the eyes (first aid: wash with clean water for at least 15 minutes and, if possible, consult a physician). Care should be taken not to over-inject and bloat the specimen to unnatural proportions. Avoiding bloat is especially tricky with very small specimens, which tend to expand noticeably even when tiny volumes of fixative are injected. Consequently, small reptiles are much more difficult to preserve well than are larger specimens. Bloat can be minimized in small specimens by using concentrated fixative (15–20% formalin) instead of routine 10 percent formalin. Investigators should experiment to determine the range of fixative concentrations that preserves best without putrefaction, and yet causes minimal shrinkage.

As every herpetologist knows, snakes are the most difficult reptiles to fix really well. They must be injected at numerous sites in the body cavities and in the dorsal, caudal, and cephalic musculature. The hemipenes in male squamates should also be everted (see Box 5, "Hemipenis Preparation"). Some workers also slit the ventral skin of the body and tail of snakes and some lizards (particularly the autotomous tails of certain species) so that fixative can penetrate. Short incisions (1–30 mm, scaled to specimen size) allow fixative to penetrate but prevent the viscera from bulging through the slit. Scale characters of taxonomic importance should not be incised. Ventral incisions are also the best way to fix specimens so small that they would be damaged by even a very small hypodermic needle. Piercing the body or tail skin of small specimens with the tip of a scalpel blade or hypodermic needle can also facilitate the entry of formalin.

After injection, the specimen is positioned on paper towels in a hardening tray. Plastic food storage tubs or similar dependably leak-proof containers with locking lids (e.g., Tupperware, Rubbermaid) make excellent hardening trays. The tray is lined with white paper towels that are dampened with fixative. Specimens are positioned in the tray and covered with fixative of the same type used to inject them (but do not exceed 10%

BOX 5 HEMIPENIS PREPARATION

Christopher J. Raxworthy

The structure of the hemipenes—the paired eversible copulatory organs of male squamates—is an important taxonomic character (see Fig. 25, in Chapter 6, and Figs. 45 and 47, in Chapter 10, "Determination of Age, Sex, and Reproductive Condition"). Hemipenis morphology is complex and sometimes varies markedly among closely related species and genera. Thus, hemipenis characters are frequently used to identify squamates, particularly sibling species that may be difficult to separate by other morphological characters. Dowling and Savage (1960) presented an excellent description of hemipenis morphology and the history of hemipenis research.

The hemipenis is best examined when everted because an everted organ exposes many more characters that can be studied than does an inverted one. In addition, most recent publications describe the everted organ. The best time to evert the hemipenis is during initial fixation, and it is most efficient to process all of the adult male specimens in a day's collection together. Adult male squamates can frequently be identified by the presence or differing development of secondary sexual characters such as dimorphic coloration, size, femoral pores, tail length, or swelling at the base of the tail. The hemipenes of juvenile and nonbreeding males may be poorly developed or impossible to evert. In life, the hemipenis is everted by contraction of a propulsor muscle (with relaxation of the retractors) and entry of fluid into the blood and lymph sinuses of the hemipenis wall. These actions cause the internal hemipenis sac to turn inside out, extending through the cloacal opening, and to pull the anterior end of the retractor penis magnus muscle into the core of the organ (Dowling and Savage 1960).

The tail muscles of a euthanized specimen must be completely relaxed for the hemipenis to be everted successfully. Pentobarbital-euthanized snakes are sufficiently relaxed about 1 hour after euthanasia (Myers 1974). In my experience, an hour is also about right for larger snakes and lizards (>100g) that have been euthanized by injection of saturated chloretone (Chlorobutanol, or trichloro-2-methyl-2-propanol) solution or other drugs, see "Euthanasia," above). The simplest way to evert the hemipenes is to inject fixative on each side at the base of the tail, just posterior to the inverted hemipenes. The pressure of the fixative will usually push the hemipenis out, although with large specimens a surprisingly large amount of pressure may be needed. Safety goggles and a syringe with a Luer lock should be used because formalin is particularly likely to squirt randomly during this operation. The hemipenis should be everted carefully until the tip is fully visible. The presence of a central dimple in the end of the hemipenis means that eversion is incomplete. However, preparators should be careful not to rupture or distort the structure by over-eversion. The hemipenes of large specimens should be tied off at the base; this practice will help to prevent their partial re-inversion if the retractor muscles contract during fixation. Retractor muscles can also be cut to prevent inversion.

If the hemipenis does not immediately begin to evert, exerting pressure with a finger just posterior to the position of the hemipenis while continuing the injection may help. This may also cause the paired anal sacs or the tiny diverticula (female structures homologous to male hemipenes; Arnold 1984) to evert, but those structures are so much smaller and morphologically simpler than hemipenes that they are not likely to be mistaken for them. If injection and pressure do not work, the investigator can make a small incision in the ventral surface of the tail posterior to each hemipenis and cut the longitudinal retractor penis magnus muscle. This muscle is attached posterior to the inverted organ and is usually obvious if the ventral skin is peeled back. For bifurcate (divided) organs, both arms of the muscle are cut. The investigator then uses a large blunt hypodermic needle to inject fluid into the tail base to evert the hemipenis. The needle is pushed forward (from the incision) toward the cloaca, and the thumb is pressed at the base of the needle to seal the cavity around the hemipenis and contain the injected fluid. The hemipenis can also be everted by injecting directly into the blood sinus at the base of the organ (Dowling and Savage 1960). Cutting the retractor penis magnus muscle is the best way to prevent partial hemipenis inversion during fixation. Partially collapsed hemipenes can be fully everted in the laboratory by injecting hot paraffin wax into the organ.

I prefer to evert both hemipenes because the two may differ morphologically (Dowling and Savage 1960). However, some researchers (e.g., Myers 1974) prefer to evert just the right organ so that the inverted (left) organ can also be studied. If an everted hemipenis is severed from the body, information on organ-body orientation is lost, and, more importantly, the isolated organ may become disassociated from the fixed specimen (especially in the field).

formalin or 70% ethanol). The fixative and specimens are covered with more paper towels, and the hardening tray is closed securely. Lizards and small crocodilians are usually fixed in a "relaxed" position (straight tail, legs close to the body, toes spread as much as possible), although one often sees lizards preserved with their tails doubled alongside the body. The problem with the latter method is that it is difficult to remove a single specimen from a jar full of similarly preserved lizards without breaking tails and otherwise damaging specimens. On the other hand, lizards with total lengths greater than 30 cm generally must be preserved with the tail doubled alongside the body to fit into standard storage containers. Snakes and large legless lizards should be coiled or looped (Fig. 29) in the hardening tray. Alternatively, a medium- to large-size snake (900–1400 mm total length) can be looped in a tray or coiled "belly up" in a gallon jar and just covered with fixative, which will help to hold the shape. Coiled snakes are stored in jars one on top the other; looped snakes stand on end beside each other and occupy somewhat less space in the jar. When snakes are coiled, the spiraled layers often sit atop one another and must be separated and held apart to provide an unobstructed view of the ventral scales. Ventral scales on looped snakes, in contrast, can be examined and counted directly. Snakes should be fixed with the mouth propped open (with a wooden stick or hard wad of cotton) to facilitate later study of the teeth. Turtles are positioned with the legs, tail, and head fully extended and the mouth propped open.

The tray is kept covered, and specimens usually harden within 12 h, but fixation continues for at least a week (Pearse 1980). The preparator should replace the fixative at least once during the initial 24 h with twice the original volume, if possible. After 1 to 2 days in the hardening tray, a hardened specimen is transferred to a storage container and immersed in bulk fixative. The fixative volume per specimen decreases as more specimens are added to the storage container, so the fixative may need to be changed periodically until transport home. This is important because weak fixative does not preserve well. Uneven hardening and preservation can largely be avoided by keeping the storage container tightly closed and not adding "used" fixative from the hardening tray when transferring specimens to the storage container. A periodic check of the specimens should reveal any problems. Polyethylene 5-gal tubs with locking lids and neoprene gaskets make excellent field specimen-storage containers. Hardening trays and storage containers should always be covered. Some herpetologists prefer to wait until after initial hardening to tag their specimens, because attached tags may interfere with proper specimen positioning in the hardening tray (McDiarmid 1994). In all such instances investigators must be especially careful to keep track of their specimens and tags. One way to do that is to lay the tag across the specimen before covering it with paper towels.

Specimen fixation in ethanol follows the same steps as fixation in formalin, but ethanol-fixed material usually takes longer to harden, and it must be monitored closely for signs of putrefaction. Simmons (2002) recommended always incising ethanol-fixed specimens after injection. Specimens destined for clearing by alkali followed by skeletal staining should be eviscerated through a large ventral incision, injected with 70 percent ethanol, and immersed in 70 percent ethanol. The brain can be removed (prior to alcohol injection and immersion) by tilting the head forward, cutting the neck skin and connective tissue transversely behind the skull to expose the foramen magnum, and injecting 70 percent ethanol into the foramen magnum to flush out the tissue.

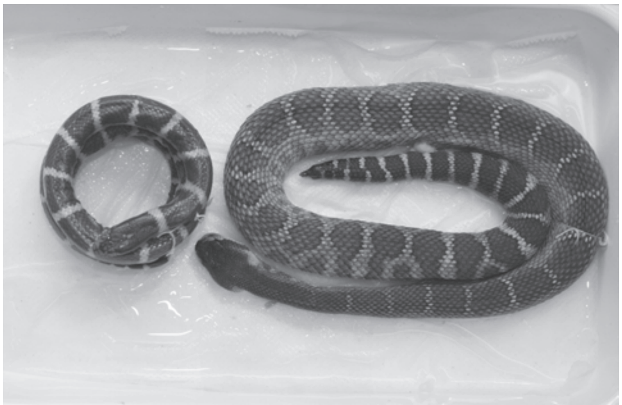


FIGURE 29 Hardening tray showing preserved specimens of (left) a coiled Common Kingsnake (*Lampropeltis getula*) and (right) a looped Western Rattlesnake (*Crotalus oreganus*). Note that the looped specimen lies flat. In the coiled snake, two or three coils lie atop one another.

Identifiable food masses in reptiles are valuable for research on diets and as vouchers for prey species. Large food masses will putrefy *in situ* in preserved specimens, so they must be removed and preserved separately. Palpating visible food masses in living snakes causes the snake to regurgitate. Likewise, a simple pumping device can be used to flush out the stomach contents of live turtles and juvenile crocodiles before release or preservation (Legler 1977; Fields et al. 2000). Large food items from dead squamates can be recovered through the same incision that is often used to obtain a sample of liver tissues for molecular studies (see "Standard Procedures for Collecting and Preserving Tissues for Biochemical Analyses," below).

Food masses should be preserved in formalin (as described above) and catalogued and tagged to reflect their origins. Multiple small food items from a single specimen can be preserved together in a tagged cheesecloth bag or special histology pouch, always associated by collector's field number with the originating specimen.

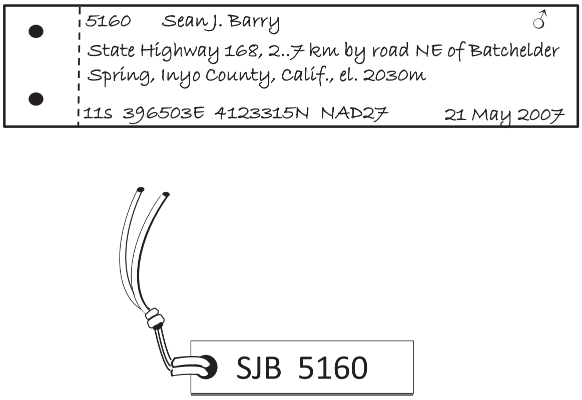
Most gravid female reptiles can be fixed in routine fashion, although barbiturate injection to the mother may not always kill near-term young of viviparous species. Large eggs and young can be fixed by injection *in situ*. However, unless a special reason exists to leave large eggs or embryos in the specimen, it is best to remove them from the female and preserve them separately, using a single collector's number for the mother and her offspring (properly noted in the catalogue). For methods of removal and preservation of embryos, see "Collecting and Preserving Embryos," below. At least one egg from any clutch discovered in a deposition site should be measured and then preserved by direct immersion in buffered 3 percent formalin (Anderson 1965). Stronger formalin tends to shrink and wrinkle soft-shelled reptile eggs. One or two others can be incubated and preserved later, either after hatching or when hatching is imminent. If it is desired to preserve near-term young within the eggs, they should be euthanized with pentobarbital and fixed with 10 percent formalin *in situ,* and the intact egg should be immersed in 10 percent formalin. It is important but obviously difficult to monitor injections and the progress of hardening in a young squamate or turtle concealed within an egg shell. Viable eggs can be incubated in a closed polyethylene plastic bag half-full of moistened vermiculite or other mold-inhibiting, moisture-retaining substratum. The eggs sit on or slightly in the substratum, and the bag is suspended in indirect but bright light away from drafts and maintained at a stable temperature and humidity as manifested by condensation on the sides of the bag (Zweifel 1961).

TAGGING SPECIMENS

Preserved specimens are nearly worthless unless they carry permanent tags that reference or include accurate data. This process begins in the field. After recording relevant data in the field book and assigning a field number to a specimen, the collector attaches a permanent tag made of heavy waterproof paper preprinted or (preferably) embossed with the field number (and sometimes the collector's initials) to each specimen (Fig. 30). Heavy waterproof paper is the only acceptable choice for these tags. Metal tags can corrode in fixative, plastic tags can become brittle and break, and other types of paper smear or tear easily. Associated data, identified by the field-catalogue number, are recorded in the personal field catalogue of the collector and entered into the collection catalogues of the institution where the specimens will be deposited. Waterproof number tags can be purchased in preprinted series from suppliers such as Allen-Bailey Tag and Label or National Tag. Such tags are worth the expense because they are durable, remain legible, and eliminate inadvertent duplication of numbers. They are typically punched with a single hole and threaded with a spacing knot (Fig. 30. Labels are tied around the necks of legless reptiles and around the right hind leg above the knee of other reptiles, always with a double surgeon's knot or square knot.

Instead of using a number tag, collectors from some institutions attach a handwritten data tag to each specimen, noting the collector, field number, date, and locality, and sometimes other information. Although this practice is not uncommon, it has significant drawbacks, and I recommend the use of a single, small number tag only. The larger data tags can catch on each other when multiple specimens are stored together and often must be untangled before a specimen can be accessed; they are also more likely to be lost or possibly to disintegrate with time, even tags made of the highest-quality rag paper. Some collectors use the larger data tag for the first specimen in a series of the same species with the same collection data (date, locality, etc.) and number-tag that and all subsequent specimens in the series. This practice seems unnecessary, particularly because specimens in a series are not necessarily stored together (depending on their size and number) or because they may be distributed among several museums. In addition, the associated data are duplicated in the collector's catalogue and the catalogue of the museum where the specimens are deposited.

If data tags are used, the quality of the tag paper, ink, and the thread used to attach the tag to the animal is important because the tags must survive and remain legible after decades of immersion in preservative and periodic or even frequent handling. Institutions that use such a system typically use tags made of white Resistall (or other 100% rag paper), with data written with Higgins Eternal ink and attached with heavy white 100 percent linen or cotton thread. This combination has proven as durable as can be expected from such a system. Higgins Eternal ink is a carbon-particle suspension in water that resists fading even after long-term alcohol immersion. It is by far the best choice for wet-specimen data tags. This ink can be used in technical pens, fountain pens, or crow-quill pens, but the bottle must be shaken vigorously before each use to suspend the carbon particles before filling the pen. India inks and most other "permanent" inks tend to flake or fade and should not be used on "wet" tags. A pencil can be used if Higgins Eternal ink is not available. Specimen-tag paper and thread are generally made from natural plant fibers because these resist damage from insects and most solvents and retain structural integrity indefinitely. Any heavy white 100 percent rag or linen paper may work, but fastness and durability should first be tested by rubbing the writing on a candidate label held under water. The longevity of synthetic tags and threads is unknown; some of these materials degrade after prolonged immersion in alcohol preservatives, so preparators should always avoid them. White paper is standard because pigments from colored papers dissolve in preservatives and may stain specimens. Two vertically aligned 1- to 2-mm holes are typically punched into the larger full-data tags at their "left" end; thread is passed through the holes and tied with a spacing knot so that the tag hangs free from the animal's body (Fig. 30).



5160 Sean J. Barry ♂
State Highway 168, 2..7 km by road NE of Batchelder
Spring, Inyo County, Calif., el. 2030m
11S 396503E 4123315N NAD27 21 May 2007

FIGURE 30 Specimen tags. (Top) Standard data tag, written in a standard format. (Bottom) A 6- × 25-mm field-number tag, with the collector's initials preceding one of a series of consecutive numbers.

DATA NOTATIONS

Dates can be recorded as "day month year" or as "month day year," as long as the name of the month is written out (recommended) or abbreviated with letters (e.g., 23 Sept 1979, May 5, 1997). To avoid ambiguity, months should never be designated with numbers. I recommend "day month year," because then the written month separates blocks of numbers that might otherwise be crowded together and difficult to interpret (e.g., July 22202, July 222002, Sept 2, Sep 12). Locality data are recorded in the field notes/specimen catalogue. Many workers record landmark-based locality data in a specific- to general-information format (e.g., "500 m by road ESE junction State highways 35 and 84, elev. 460 m, San Mateo County, California"); others use a general-to-specific format (e.g., "California, San Mateo County, 500 m by road ESE junction State highways 35 and 84, elev. 460 m"). Collectors should use a global positioning system (GPS) device (if available) or a topographic map to obtain latitude and longitude or Universal Transverse Mercator (UTM) coordinates at the collecting site, and an altimeter or a topographic map to obtain elevation. They should record these data in the field catalogue (and on the data tag attached to the first specimen in a series, if they use one).

Many investigators prefer to record UTM coordinates rather than latitude/longitude, because UTM coordinates involve neither decimals nor dissimilar units (degrees, minutes, and seconds) and because linear distances among a series of UTM coordinates can easily be calculated without using a map. Users of GPS devices should select the appropriate datum for the region and record it in the field notes along with the UTM zone if that coordinate system is used.

HANDLING PARASITES

Ectoparasite burden is an important ecological and systematic parameter. The entire ectosymbiont fauna from at least one specimen of each reptile species per locality should be preserved. Endosymbionts can be collected in the field, or they can be left in place in the intact (fixed) internal organs of the host, for later dissection and study in the laboratory. During fixation, the visceral cavity of any specimen specifically intended for later parasite examination should be opened with one or two long incisions so that fixative penetrates completely. Endoparasites should be recovered in the field from specimens whose viscera will be discarded. Detailed protocols for collecting parasites are covered in "Collecting and Preserving Parasites During Reptile Biodiversity Survey," later in this chapter.

OVERSIZE SPECIMENS

Formalin fixation works for virtually any reptile at least up to sea-turtle size, but it is often impractical to fix and preserve entire large specimens in fluid because of the enormous storage containers required, both in the field and at the home institution. Some large reptiles, such as varanids and medium-size boids, can be fixed without complete immersion in formalin. The specimen is injected thoroughly with 10 percent formalin, wrapped in several layers of cheesecloth and paper towels saturated with 10 percent formalin, and enclosed in a large plastic bag or "sandwiched" between plastic sheets. The fixation process must be monitored closely. The wrapped specimen is stored in a heavy polyethylene bag or wrapped in several layers of polyethylene when fixation is complete.

Only the preserved heads and skins or cleaned, dried skulls and dried skins or shells of very large reptiles (large boids, varanids, some turtles, and most adult crocodilians) are normally saved as voucher specimens. Alternatively (and preferably from both practical and conservation viewpoints), investigators can collect as vouchers only small, but identifiable, juvenile specimens of large species and preserve them by conventional formalin fixation. The head and neck of a large specimen can be severed from the carcass and injected and preserved as described previously. A large sewing needle is used to penetrate the head tissue and thread the tag around the mandible. To skin a large squamate or crocodilian, the skin is incised ventrally from neck to tail tip, and from the central incision laterally along the ventral surfaces of the limbs to the middle of the foot (some collectors leave the foot skin intact). The throat and chin skin should be left intact to avoid obscuring taxonomically important scale characters. Some workers stop the incision just ahead of the anal plate and peel the skin back from the tail. The anal plate and anal spurs (if present) should be left intact. The skin is peeled away from the tail toward the head. The skin should be worked outward along the limbs, or the limbs can be disarticulated from the girdles and skinned later after the skin is freed from the rest of the carcass. The skin is freed from the jaws, ear orifices, and eyes from the inside so that the scales are not damaged. The claws should remain attached to the skin. If the skin is to be dried, the phalanges are removed out to the base of each claw. If the skin is to be preserved in fixative, each foot is disarticulated at the tarsals or carpals and left intact inside the skin. Soft tissue is scraped from the inside of the skin with a stout knife (difficult in most pythons). The feet are injected with fixative, and the rolled-up skin is fixed in 10 percent formalin. Very large skins can be tacked to a flat surface and air-dried, then rolled loosely and packed for shipment home. Such dried material is vulnerable to insect attack, so fully dried skins should be stored in airtight containers, if possible. Most large reptiles are relatively easy to skin completely, but the head scales of some specimens adhere so tightly to the skull that they cannot be removed intact by the normal skinning process. In those specimens, the rest of the skin is removed as described above, and the loose skin is severed from the head. Maceration in ammonia or immersion in hot water at the home institution will usually loosen the remaining head scales. Very large turtles are skinned along the edges of the shell, and the forelimbs and head/neck are removed as a unit for later cleaning or for fixation in formalin. Finally, all soft tissue is removed from the interior of the shell (a very laborious and disagreeable task). Sawing through the shell bridge and removing the plastron makes this job much easier, but it diminishes the utility of the specimen.

Most museums use the larvae of dermestid beetles to clean reptile skeletal material. Material presented to a dermestid colony must be thoroughly dry and formalin-free. To ready a skull for transport home and later "bugging," the collector removes the eyes, extracts the brain by flushing the skull with water injected through the foramen magnum, and removes the large muscle masses from around the jaws and occipital region, taking care not to damage the hyoid or the thin stapes bone between the quadrate and postorbital of snakes. The skull is tagged around the mandible and then suspended in an air current and dried thoroughly. Secure tagging is especially important because the skull may be unidentifiable if it is permanently disassociated from the skin. If flies are a problem during air-drying, the skull should be wrapped in cheesecloth. Skulls can be dried rapidly by hanging them securely from a horizontal wire in the engine compartment of a truck—an hour's exposure to engine heat will dry most skulls completely. Alternatively, prospective skeletal material can be fixed and stored in 70 percent ethanol. The specimen is later soaked in water to remove the alcohol and then air-dried thoroughly so that the dermestid larvae will clean it (Hildebrand 1968). The eyes of lizards and turtles can be air-dried or fixed *in situ* or separately for later preparation of the sclerotic ossicles. If an entire skeleton is to be saved, the specimen is skinned, eviscerated, positioned as compactly as possible, tagged, and air-dried or fixed in 70 percent ethanol. Skeletons of very large specimens can be separated into subunits (e.g., several consecutively numbered lengths of a snake body skeleton), dried, and packed compactly for transport home.

Packing and Shipping

When a field trip is almost over, investigators should account for all notes, specimens, and ancillary material, make sure that all specimens are properly tagged, and prepare for the trip home. Dried skulls are wrapped individually in cheesecloth and packed in small- to medium-size cardboard boxes lined with extra padding. Small skulls are placed between cotton pads in plastic vials. Skull and parasite vials are grouped by size. Each vial is wrapped in paper and then packed with the others into cardboard boxes with extra padding all around. Polystyrene or corn-waste packing "peanuts" contained in plastic bags make excellent padding, but loose (unbagged) packing peanuts are unsuitable for padding because heavy objects tend to work through them.

Wet specimens are packed according to the distance to be traveled back to the laboratory and the mode of transportation. If the field site is within easy driving distance of the home institution, wet specimens can be transported in their 5-gallon polyethylene storage tubs or even in hardening trays, vehicle space permitting. Each hazardous material container must be labeled prominently with the name of the chemical and the word TOXIC or FLAMMABLE (or both), as applicable. Such containers should be confined to vehicle space isolated from the passenger compartment, such as the bed of a pickup truck. Leaky containers should be discarded, and large glass jars should be avoided for both storage and transport, for obvious reasons. Investigators should comply with local and DOT regulations governing the transport of hazardous material on the highway.

If a return trip involves air travel, or if bulk fixative transport is otherwise impractical, wet material (except parasites) can be repackaged without fluid fixatives. Each wet specimen is wrapped in cheesecloth, and several such specimens are placed in a "6-mil" clear polyethylene bag. Just enough fixative is poured into the bag to saturate the cheesecloth; the excess is poured off. The specimens are double- or triple-bagged. Packing a few specimens in small bags is preferable to packing many specimens in large bags. The neck of each bag is knotted or secured with a heavy rubber band, and the bag is checked for leaks. Those bags are then enclosed in a larger polyethylene bag, and the large bags are packed in heavy cardboard boxes with ample padding. Each box should contain an inventory sheet and copies of required export and import permits. Copies of these permits should also be taped to the outside of the box and, along with the address label, covered with clear packing tape. Even without fluid fixative, fixed specimens may have to be shipped as hazardous material because of their fixative content or potential flammability. The investigator should check current government and carrier rules and package identification requirements, which change frequently. Properly wrapped and sealed bags will maintain specimens safely for months, although the specimens should be returned to the fixative or to permanent alcohol storage promptly on return home.

Supplies Required for Collecting and Preserving Specimens

COLLECTING

Appropriate permits; approved institutional animal use protocol, if applicable; lizard noose; snake tongs; dipnet; turtle traps; funnel traps; 22-caliber rifle or pistol/revolver, dust shot .22 cartridges, and shotgun or other larger firearm, as necessary; headlamp, batteries, and spare bulbs; mirror; close-focusing binoculars; cloth and pierced ziplock bags; vermiculite or sphagnum peat for eggs; tight wooden boxes up to 60×45×30cm (24×18×12 in) in size; medium and large Tupperware, Rubbermaid, or other plastic containers with watertight lids; pocket notebook; series of plastic or rag-paper number tags; Sharpie permanent ink marking pens and waterproof pen or pencil; GPS device and spare batteries; altimeter; maps; compass; thermometer and cloacal thermometer; "point and shoot" 35-mm film camera or equivalent digital camera; camera batteries; slide film or memory sticks; lens cleaner and tissue.

SPECIMEN PROCESSING

Field catalogue and notebook, metric rule and tape measure; Vernier caliper; portable field scale/balance; threaded data tags and threaded number tags; acrylic tubes with drilled holes or machined slots; pencil or pen with waterproof ink; syringes and agents for euthanizing animals; scalpel; scissors; cheesecloth; snakebite kit (e.g., splints, Ace bandages), first-aid items appropriate for local venomous species, including antivenin (if available), and associated medical supplies (per the advice of a physician); digital or 35-mm film SLR camera, camera batteries, film or memory sticks, macro lens, flash unit or auxiliary lighting, tripod, and neutral-colored poster board, cloth, or other smooth background material.

PRESERVING

Tupperware or similar trays and cylindrical containers; phosphate-buffered 10 percent formalin (or commercial formaldehyde, buffer, water); 70 percent ethanol; pH paper or hand held pH meter with spare batteries; syringes with Luer locks; 18-, 20-, and 25-gauge 1- and 2-in hypodermic needles; 16- and 18-gauge blunt hypodermic needles; safety goggles; hard-walled sharps-disposal containers; camp shower bag for eyewash; neoprene gloves; white paper towels; 5-gal polyethylene tubs with locking lids and neoprene gaskets.

PACKING AND SHIPPING

Heavy cardboard boxes; fiber and packing tape; Sharpie permanent ink pens; 6-mil polyethylene bags in assorted sizes; heavy rubber bands; string; cheesecloth; fabric scissors; plastic vials (various sizes); cotton; bulk packing material (bubble wrap, cotton batting, padding, bagged "peanuts"); mailing labels and wide, clear packing tape; multiple copies of international import and export permits.

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Collecting and Preserving Embryos

Alan H. Savitzky, William A. Velhagen, Jr., and Neil Chernoff

Investigators engaged in field studies of biodiversity regularly acquire specimens that they deposit in natural history museums as vouchers or as material for future studies. The value of these specimens for systematic, morphological, biogeographical, and many other types of research is widely appreciated. In general, such collections consist primarily of specimens of adults, which usually are most representative of the species as well as the most readily collected and easily prepared. Among herpetological collections, notable exceptions to the dominance of adult material are collections of amphibian larvae and, to a lesser degree, eggs. Curiously, although the role of developmental processes has become a central focus in evolutionary studies (viz., the field of evolutionary developmental biology, or "evo-devo"; e.g., Raff 1996; Carroll et al. 2001; Jeffery et al. 2002; Shapiro 2002; Gilbert and Epel 2009), little effort has been made until recently to collect and preserve embryonic reptiles beyond a few "model" taxa. Although model species provide important opportunities for experimental laboratory studies, they represent only a minute fraction of the natural diversity of reptilian developmental patterns. In the same way that systematists eschew the building of phylogenies based upon only a handful of taxa, developmental biologists should likewise avoid basing comparative studies on too narrow a taxonomic base. This situation is slowly changing, as attention is being paid to development in additional reptilian taxa (e.g., Greenbaum and Carr 2002; Sheil 2005; Noro et al. 2009). In this section we provide information on the collection and preservation of reptilian embryos, with the hope of encouraging more field biologists to collect and preserve them. We include a discussion of the ethics of such activities and of the often unique data standards required to maximize the utility of such specimens.

In addition to illuminating the conceptual aspects of vertebrate development, collections of embryos can provide insights into environmental conditions, both natural and anthropogenic. Early developmental stages are notoriously susceptible to environmental perturbation, making embryos important indicators of environmental disturbance. Chemical and radiological agents or unusual thermal conditions may adversely affect embryogenesis, and the development of reptiles in some taxa has proven highly sensitive to xenobiotic substances. Recent studies have shown that adverse effects on the development of crocodilians and turtles can occur after embryonic exposure to environmental contaminants (Bishop et al. 1991; Sheehan et al. 1999), notably chemicals that exhibit estrogenic properties (Guillette et al. 2000). Detrimental effects of global warming on species exhibiting temperature-dependant sex determination have also been postulated (Janzen 1994). Developmental defects of unknown etiology have been described in numerous groups, including snakes (Oros et al. 1997) and crocodilians (Rainwater et al. 1999). The collection and analysis of embryos may prove to be essential both for the determination of teratogenic effects and for an understanding of the mechanisms involved in abnormal development. Such studies, however, require an appreciation of normal development in the subject taxa, which in turn requires more intensive collection and study of reptilian embryos than has occurred to date.

Embryos as Specimens

GENERAL CONSIDERATIONS

From the standpoint of embryo acquisition, oviparous and viviparous species present very different challenges, although the methods of embryo preservation and data standards are similar. For developmental studies, freshly collected and fixed material is greatly preferred over older material, which may not have been properly preserved and in which the tissue quality may have deteriorated. For example, after several years the skeletons of whole specimens stored in unbuffered preservative may decalcify. In addition, embryos that have been carefully isolated from surrounding structures before they are preserved are preferable to specimens obtained from previously preserved gravid females or from eggs. Specimens fixed *in ovo* or *in utero* generally are distorted by the pressure of the adjacent yolk and often suffer from delayed diffusion of fixative through the shell or maternal tissues. For some procedures, it may be advisable to culture living embryos *in vitro*, a technique to which some reptilian embryos are readily adaptable.

The size of the sample of embryos desired will vary depending upon the study being conducted. Nonetheless, some generalizations can be made, especially when embryos are collected for subsequent, unspecified developmental studies. The number of clutches or broods available and the fecundity of a species often will dictate the number of embryos that can be collected. In anoline lizards and geckos, for example, small clutch sizes (1–2 eggs) limit the number of embryos that can be obtained without establishing a breeding colony. In contrast, clutches or broods of marine turtles, crocodilians, and large-bodied viviparous natricine snakes are large (25–100 or more) and can provide substantial numbers of embryos. Under ideal circumstances, three factors should be considered in determining how many embryos to collect and preserve. First, the number should be adequate to represent the various stages of normal development and the variation within each stage. Second, it is desirable to preserve embryos of the same stage using various fixatives, to facilitate future examination using different techniques (e.g., gross dissection, histology, electron microscopy). Third, enough embryos of a given stage should be obtained so that some can be preserved as archival vouchers to document the gross morphology and staging criteria used for specimens that have been altered by preparation (e.g., sectioning, clearing and staining). In reality, only a few model taxa are likely to be represented in museum collections by large numbers of embryos. For the majority of species, collection of any available embryos will constitute an important contribution to future studies of reptilian embryonic development.

OVIPAROUS SPECIES

Oviparous species have several advantages over viviparous ones in the collection of embryos. Eggs are easily handled, and surgical excision of eggs from the dam generally is not required. However, squamate eggs usually begin to develop *in utero* and complete about one-third of their development prior to oviposition (Shine 1983). Therefore, surgical removal of eggs (Sanger, Losos, and Gibson-Brown 2008) or hormonal induction of oviposition may be required to obtain early-stage embryos of squamates. Skeletogenesis generally begins after the normal period of oviposition in squamates, so studies of skeletal development can be performed on embryos obtained from naturally laid eggs. This is not the case, however, with some other organogenetic studies, such as those dealing with the early development of sensory, neural, or somatic tissues.

Because eggs can be obtained without recourse to surgery, the reproductive status of the dam is not affected. That is an important consideration when dealing with an ongoing breeding colony or with material obtained from zoos. Unfortunately, gravid females of oviparous species generally are encountered less frequently in the field than are those of viviparous species, simply because oviparous females carry the eggs for only a portion of the total developmental period. When gravid females are encountered in the field, they can be held until the eggs are laid, and the embryos can be processed easily to obtain a developmental series. In addition, clutches of reptilian eggs discovered in the field can be used to obtain embryos, provided that at least one embryo is allowed to hatch or to reach a stage of development late enough to ensure the identity of the species unambiguously. Finally, clutches of eggs provide a known quantity of embryos (assuming a high rate of fertilization), so plans for obtaining a developmental series can be carefully established before the harvesting of embryos begins.

VIVIPAROUS SPECIES

Embryos of viviparous species spend a longer period *in utero* than do those of oviparous species. Consequently, gravid females of such species are more likely to be encountered in the field and, thus, are more likely to be found among existing museum specimens. Although embryos from such specimens usually are not perfectly preserved, they nonetheless may be the only embryos available for many less common species.

In viviparous species the number of progeny in a brood generally is estimated by palpation, although ultrasound or X-ray techniques can be used to obtain a more precise count of embryos *in utero.* Obtaining fresh embryos from viviparous species requires surgical excision (i.e., caesarean surgery). That procedure may render the female nonreproductive, a strong disadvantage when working with a captive breeding colony. In contrast to the embryos of oviparous species, however, those of viviparous species are readily cultured *in vitro.* For gravid females captured under field conditions, it is generally impractical to perform sequential surgeries (see “Viviparous Species,” under “Collection of Embryos,” below) to obtain a developmental series. If a female cannot be returned alive to the laboratory, the only option may be to collect and prepare the entire series of embryos simultaneously, after the female has been euthanized. Although such a procedure generally yields embryos of only a single developmental stage, the quality of the embryonic material is maximized. If several gravid females of a species are collected simultaneously, it may be possible to maintain some alive for longer periods, to increase the likelihood that a range of embryonic stages will be obtained.

Collecting and Processing Embryos

OVIPAROUS SPECIES

Collection of embryos from oviparous species generally begins with maintenance of a gravid female. A suitable environment for oviposition must be provided; for most species, that requires a situation sufficiently humid to prevent embryonic death from desiccation of the eggs, which can occur within a few hours. An oviposition container with damp sphagnum moss, which retains moisture for weeks while retarding bacterial and fungal growth, is sufficient. If the eggs are adherent, like those of many squamates, an attempt should be made to separate them gently if they are discovered soon after oviposition. Separate eggs are easier to open later, and the transmission of bacterial or fungal infections is easier to control. For some taxa, such as turtles and crocodilians, it is important to avoid turning the eggs, which can damage membranes and compress the embryo under the weight of the yolk. The eggs of squamates, however, generally can withstand turning.

Eggs should be incubated at a controlled temperature and high humidity (essentially saturated). Clear plastic containers with reasonably tight-fitting lids work well when used in a laboratory incubator. A good substrate consists of relatively fine vermiculite, thoroughly moistened but not wet (a mixture of equal parts vermiculite and water by mass works well). Bacterial and fungal growth can be greatly reduced by autoclaving the vermiculite before use. Recently, vermiculite has become relatively difficult to obtain in the United States, owing to human health concerns; perlite makes a suitable substitute. It is very dusty, however, and (as with vermiculite) care should be taken to avoid inhaling the dust. Perlite also is very light and appears to hold more water per unit weight than vermiculite. Water should be added slowly to perlite to avoid oversaturation. A correct level of saturation is achieved when the perlite is uniformly moistened (i.e., it does not form separate clumps) but free water is not evident. Perlite can also be autoclaved, but it does not appear to support microbial growth as readily as vermiculite, so sterilization may not be required.

Although under natural conditions the temperature of the oviposition site may vary on a daily and seasonal basis, a single standardized temperature ensures uniform conditions for comparative studies of developmental rate. An incubation temperature of 30°C is suitable for most temperate and tropical squamates. In most turtles, crocodilians, and *Sphenodon,* and in a few lizards as well, sex is determined by developmental temperature (Pough et al. 2003). Therefore, the incubation temperature must be carefully selected for these taxa, depending upon the desired sex ratio of the progeny.

Under field conditions, where supplies may be limited and incubation conditions difficult to control, moist rotting wood or damp paper towels may be used to incubate reptilian eggs. A plastic bag can be used as a container if a lidded box is unavailable. Eggs should be checked regularly for bacterial and fungal growth, which usually begins after an embryo dies and progresses rapidly. An infected egg should be promptly removed, and the contained embryo checked to determine whether it is still suitable for preservation and study.

When collecting a synoptic developmental series from a species for the first time, we recommend that at least one egg from each of the first few clutches be opened on the day of oviposition (designated Day 0), to determine the usual stage at oviposition. We also recommend that at least one egg from each clutch be left to hatch, to determine the total length of the incubation period under the conditions used. For species that have been studied for some time under controlled conditions, these conventions can be discontinued.

The use to which the embryos will be put will determine the schedule of preservation for each clutch of eggs. Some studies may require primarily early embryos, whereas others will require mostly later stages. For collection of a representative embryonic series, the number of eggs available in each clutch must be compared with the estimated incubation period (derived from data on related taxa) to determine a schedule for opening the eggs and preserving embryos. Because embryos change most rapidly early in the incubation period, when primary organogenesis is taking place, it may be desirable to preserve embryos more frequently during that time. Other developmental processes, such as ossification of many skeletal elements, proceed slowly throughout later embryogenesis, even though the embryos pass through few described stages of normal development (which are based primarily on external characters) during that time.

Before removing an embryo from the shell, it is often helpful to candle the egg—that is, to hold it up to a strong point source of light to locate the embryo. A fiber-optic light shining through a narrow aperture is effective. (An alternative method of detecting the position of an embryo in a small egg, such as those of *Anolis* spp., is to transilluminate the egg from the side and observe the embryo's shadow; Sanger, Hime, et al. 2008). The shell is then cut with fine scissors near the embryo to produce an opening large enough to remove the egg's contents. The embryo is carefully dissected from the yolk and extraembryonic membranes. It is often helpful first to empty the egg into a small dish, separate the yolk from the embryo, and then transfer the embryo to a second dish, where it can be removed from the amnion using fine forceps or scissors. This procedure reduces the likelihood of contamination of the embryo with yolk. After recording any necessary measurements, the embryo is placed in fixative. The unfixed embryo must be handled with great care, using forceps or a small spatula, to avoid damaging the delicate tissues.

VIVIPAROUS SPECIES

For viviparous species, fresh embryos (Fig. 31) must be obtained by surgical excision. If embryos of different stages are to be obtained from a single female, sequential surgeries will be required. The number of surgeries that can be performed on a single female varies with the number of embryos and the size and condition of the dam. We have successfully performed up to five sequential surgeries on large snakes with broods of more than 20 embryos, with some embryos being left to develop to full term *in utero*. We recommend that a minimum of 2 days be allowed for recovery between surgeries. (If more closely spaced embryonic stages are required from a single female, *in vitro* culture of some of the embryos is preferable to more closely spaced surgeries; see "*In Vitro* Embryo Culture," below).

Surgeries must, of course, be performed under anesthesia (McDonald 1976; Frye 1991; also, see "Anesthesia in Reptiles," in Chapter 8, "Dealing with Live Reptiles"). An injectable anesthetic agent, such as sodium pentobarbital, may be used, but we have found that inhalant anesthetics are relatively safe, convenient, and easy to monitor, and they have relatively rapid recovery times. Isoflourane and halothane are affordable inhalant agents and are effective for many reptiles. At the ideal level, the subject is fully anesthetized but still breathing. If breathing is suppressed, ventilation can be maintained by blowing or pumping air into a catheter inserted in the glottis. Such ventilation can also be used following surgery to speed recovery from anesthesia. Rapid clearance of the anesthetic agent from tissues is especially important when sequential surgeries are to be performed, in order to avoid prolonged exposure of the remaining embryos.

For most squamates, surgical excision of embryos requires a parasagittal incision through the ventral body wall to avoid severing the ventral abdominal vein (see Frye 1991). Once the peritoneal cavity is open and the oviducts are exposed, embryos can be removed by either opening the oviduct or completely removing a section of the duct. Opening the oviduct with a longitudinal incision allows the embryo to be removed with the oviduct still largely intact. The extraembryonic membranes likely will be damaged during that process, risking leakage of yolk into the peritoneal cavity. Furthermore, the oviduct must be surgically repaired, with little assurance that it will be returned to full function. Unless it is essential to return the female to reproductive capability and skilled surgical expertise is available, we recommend removing embryos by excising a section of the oviduct with the embryos contained therein. After determining the number of embryos to be collected, the anteromost section of the oviduct is ligated with suture material posterior to the series of embryos selected. If sequential surgeries are performed, they involve progressively more posterior sections of each oviduct. It may also be necessary to ligate some of the larger blood vessels serving the excised region of the oviduct. The selected oviducal section and its contained embryos are then removed. Individual embryos are cut from the oviduct and then separated from the membranes, preferably using two dishes, as described above for embryos of oviparous species.

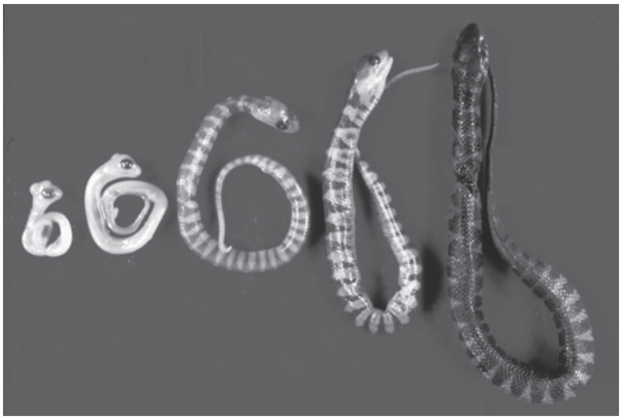

FIGURE 31 Embryos of the Southern Watersnake (*Nerodia fasciata*) representing Stages 32, 33, 35, 36, and 37 of development, according to the staging system of Zehr (1962). Embryos were removed surgically from the dam, separated from the extraembryonic membranes and yolk sac, and fixed in 10 percent phosphate-buffered formalin. (Photo by W. A. Velhagen, Jr.)

If embryos are to be removed from existing museum specimens, a similar procedure is followed. Midventral incisions through the ventral body wall and oviduct are used to expose the embryos. The latter should be removed gently, as they frequently become brittle during preservation and storage.

IN VITRO EMBRYO CULTURE

Embryos can be maintained *in vitro* either to obtain closely spaced embryonic stages or to subject the embryos to experimental procedures that would be difficult or impossible to conduct *in ovo* or *in utero*. Although a procedure for experimental manipulation of turtle embryos maintained in "capped" (opened) eggs has been described for the hard-shelled eggs of turtles (Yntema 1964) and geckos (Noro et al. 2009), we are unaware of any attempts to remove reptilian embryos from the egg and culture them *in vitro*. Although such a procedure has been applied to chick embryos (Auerbach et al. 1974), it is difficult to extract the contents of a shelled egg intact, especially from eggs with flexible shells. Therefore, *in vitro* culture of reptilian embryos has been limited to viviparous squamates (Holtzman and Halpern 1989).

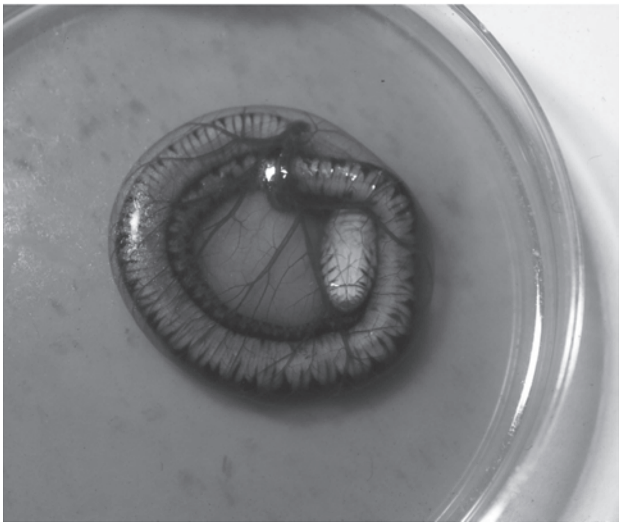

FIGURE 32 *In vitro* culture of an embryonic Southern Watersnake (*Nerodia fasciata*). The embryo was removed surgically from the dam and placed in a Petri dish with tissue culture medium. Note intact yolk sac, chorioallantoic membrane, and vasculature. (Photo by W. A. Velhagen, Jr.)

A procedure described for culturing embryos of *Thamnophis,* a natricine snake (Holtzman and Halpern 1989), is adaptable to many other viviparous taxa. Each embryo is placed in a sterile dish of suitable size (deep plastic Petri dishes work well for smaller embryos), and sterile tissue culture medium (e.g., Minimum Essential Medium Eagle's) is added to about half the depth of the egg (Fig. 32). Although the oviduct can be cut away from the embryo, we have found that if the embryo is placed in the culture dish with the surrounding oviducal tissue attached, the latter usually shrivels up in several hours and falls away, leaving the extraembryonic membranes intact. The medium can be supplemented with a broad spectrum antibiotic-antimycotic drug (e.g., a penicillin-streptomycin solution) and various growth factors (e.g., chick embryo extract, which provides an array of factors). However, we have found that unmodified commercial tissue culture medium works well for culture periods of several days. The culture dishes are placed in an incubator at a suitable temperature (30°C works well for many species). No special atmosphere is required for culturing reptilian embryos. With this procedure we have routinely achieved survival periods of 3 to 7 days. Culture periods greater than 14 days were rare, with embryos generally succumbing to infection. Cultured embryos may be smaller than those of similar age that gestate in the female (Velhagen 1995).

Handling and Storage

MEASUREMENT

If possible, specimens should be measured before fixation. Standard measurements include snout-vent length, tail length, total length, body mass, and residual yolk mass. It may be difficult to obtain length and mass of young embryos, because of their small size; adherent yolk, tissue, and fluid; and, in snakes, coiled body. Although length can be measured post mortem, mass taken at that time may be inaccurate because of osmotic gain or loss of fluid. If the mass of residual yolk is large relative to the mass of the extraembryonic membranes, it is possible to weigh the egg's contents before and after removal of the embryo and then obtain an approximate residual yolk mass by subtraction. With embryonic measurements, the rate of embryonic growth can be calculated (Velhagen and Savitzky 1998), and it may be possible to estimate the total length of the incubation or gestation periods for strongly lecithotrophic taxa. Embryonic body mass and residual yolk mass together can also provide an estimate of parental investment for studies of reproductive ecology.

LABELING AND STORAGE

Embryos should be numbered individually and labeled at the time of collection (even if they are to be cultured *in vitro*). Fluid-resistant tags printed or handwritten in permanent ink are required. Multiple siblings that are removed simultaneously and prepared identically can be stored as a lot and assigned a series of numbers; individual numbers from the series can later be assigned to specific embryos as the latter are prepared for individual study. If embryos are measured at the time of collection, however, they must be individually numbered immediately. Tags can be tied onto larger, older embryos, but smaller, younger ones may be too fragile for the tags to be attached. In that case, each embryo and its tag should be placed in an individual vial.

Embryos can be both fixed (see "Fixatives," below) and stored in the same fluid and in the same container. The body cavity of older embryos should be opened or injected to ensure penetration of fixative. Likewise, late-stage embryos may require euthanasia prior to fixation (Sanger, Hime, et al. 2008). Body mass must be recorded before a euthanizing agent or fixative is injected, to avoid the confounding effects of the fluid mass. A specimen should be stored in a volume of fixative that is at least 10 times the volume of the specimen.

A variety of containers are available, but glass vials with polyethylene snap caps (e.g., Wheaton) are fluid-tight and reasonably reliable for most preservatives (Fig. 33A). Polypropylene tubes of various sizes have also been recommended (Sanger, Hime, et al. 2008). Screw-cap vials are often less reliable than snap caps, although the addition of Parafilm under the cap reduces evaporation. For archival storage, placing the vials in plastic boxes with tight-fitting lids (Fig. 33B, C) and storing the boxes in cabinets with gaskets that form a tight seal (herbarium cabinets work well; Fig. 33D) minimizes evaporation of fixative and leakage of fumes. Storage containers must be checked periodically to guard against evaporation from vials with faulty closures.

FIXATIVES

The choice of fixative is determined largely by the anticipated use of the specimens. A standard, general-purpose fixative and preservative is 10 percent phosphate-buffered formalin (PBF; Presnell and Schreibman 1997). Buffering is essential to prevent the acidic degradation products of formalin from decalcifying the bones of the embryo. Specimens fixed in PBF are suitable for gross dissection, routine histological preparations for general studies of microanatomy, and clearing and staining for studies of bone and cartilage.

Another useful fixative for some histological procedures, including the use of certain silver stains, is formalin-acetic acid-

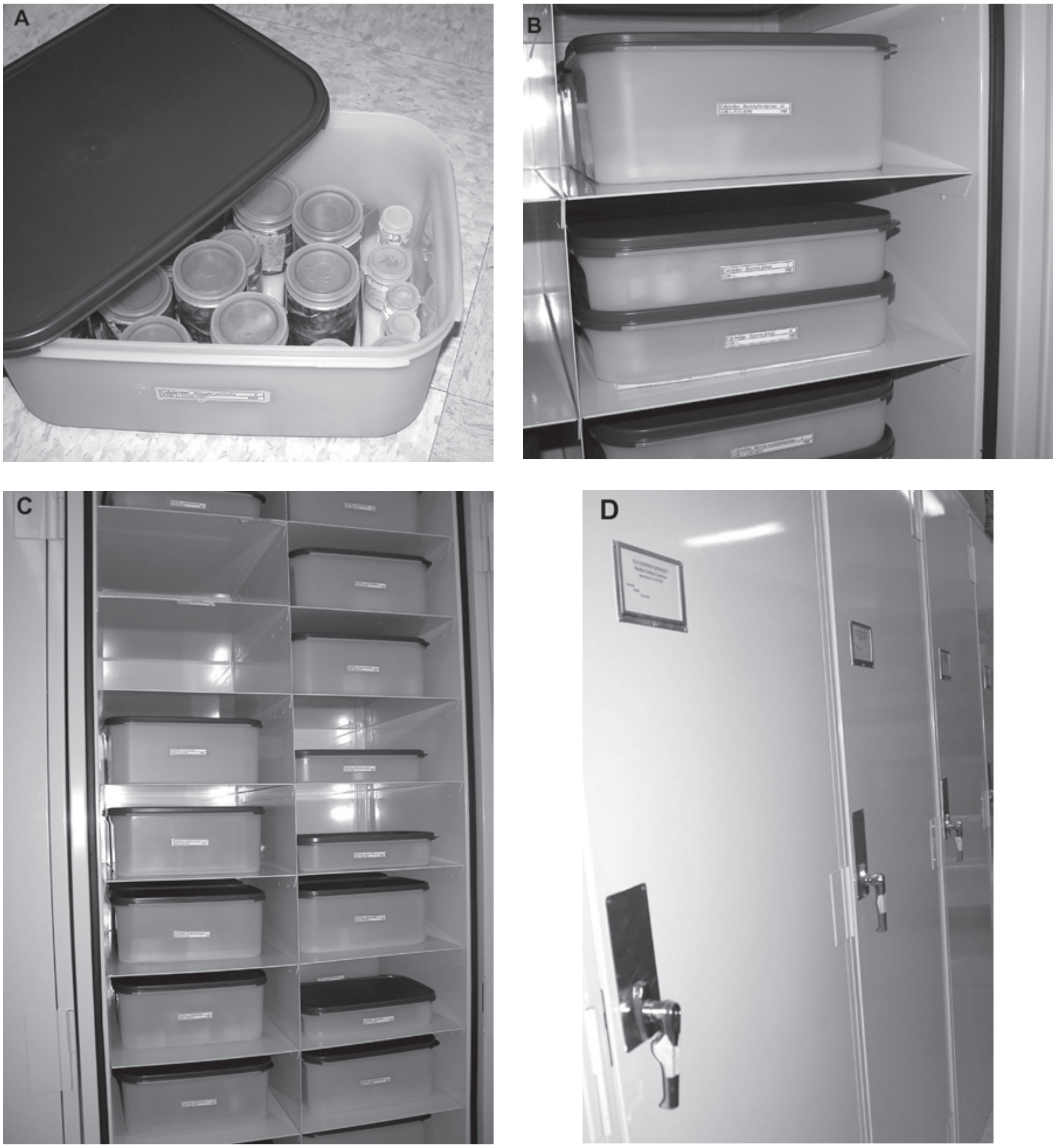

FIGURE 33 Storage for specimens of embryos. (A) Embryos in snap-cap vials (Wheaton) placed inside a plastic storage box (Tupperware). (B) Plastic boxes of two depths, accommodating vials of different sizes, on shelves of a herbarium cabinet. Interior (C) and exteriors (D) of herbarium cabinets used for storage of embryos. Placing the vials inside plastic boxes and storing the boxes in a tightly sealed herbarium cabinet minimize evaporation of preservative and leakage of fumes into the surrounding workspace. (Photos by A. H. Savitzky.)

alcohol (FAA; Lewis et al. 1981). FAA penetrates tissues relatively rapidly and turns young embryos an opaque, ivory white (rather than the translucent preparation that results from PBF). The opacity allows the surface structure of embryos to be seen and photographed more easily under a dissecting microscope. However, tissues fixed in FAA are more rigid and slightly more brittle than those preserved in PBF. Dent fixative is a specialized solution used for certain whole-mount immunohistochemical staining procedures (Klymkowsky and Hanken 1991). Gluteraldehyde is a standard fixative for transmission electron microscopy (TEM) and is useful when fixation of cellular detail is critical. Gluteraldehyde is often combined with formaldehyde (mixed from paraformaldehyde, to avoid contamination from preservatives) to improve its limited penetration. It also may be followed by secondary fixation with osmium tetroxide to fix lipids. Hayat (2000) has provided details concerning concentrations and buffering of gluteraldehyde, as well as additional citations regarding fixation of biological materials for electron microscopy. Note that specimens prepared for TEM generally must be taken immediately through the entire sequence of fixation, infiltration, and embedding. There is no evidence that fixation of entire embryos with gluteraldehyde, followed by long-term storage in fluid, yields acceptable TEM preparations. Instead, TEM studies of embryos require that each embryo begin the complete sequence of processing immediately after harvest.

Data Standards

The data standards for embryo collections are somewhat different from those for routine field collections. That is true not only for the embryos themselves, but also for the dams. A complete list of data fields for dams is provided in Table 8 and for embryos in Table 9. Many of these fields do not apply to specimens obtained in the field, but only to those from a breeding colony. In this section we draw attention to those data fields likely to be least familiar to field biologists.

MOTHERS (DAMS)

In addition to routine collection and accession data (e.g., locality, date, collector), data for dams ideally includes length and body mass, as well as the temperature at which the dam was maintained (if kept captive until oviposition or parturition). For each clutch or brood, additional data associated with the dam should include the identity of the sire and date of mating (generally available only in the case of captive breeding programs); date of oviposition, hatching, or birth; and clutch or brood size (i.e., total number of progeny).

EMBRYOS

In addition to the dates of oviposition and hatching or of birth, data for each embryo should include the identity of the dam and sire (if known); date of preservation; incubation or gestation temperature (if controlled); length and body mass at time of preservation; and methods of euthanasia, fixation, and storage, as well as any special methods of preparation or experimental procedures. Embryos can be staged either during or after harvest, but the thorough recording of staging data is essential. These data include the staging sequence used (generally a citation to a publication or, for well-known staging tables, simply the author), stage of embryo, and key criteria used to assign a given embryo to a particular stage (to allow subsequent workers to verify the staging assignment). Annotations should be made regarding the availability of photographs or illustrations of a given embryo or parts thereof. The morphological criteria for determining developmental stage in a given species are presented in a table of normal development, and such sequences have been described for various reptilian species. Billett et al. (1985) presented a comprehensive review of the publications available at that date; we list selected publications with major tables of normal development in Table 10.

For many reptiles, determining the absolute age of an embryo is impossible, even when the dates of mating or oviposition are known, because of sperm storage or embryonic diapause. In some well-studied taxa, however, it may be possible to calculate an approximate starting date for embryonic development, using a regression of known dates against embryo size. That calculated date is referred to as the baseline date for an embryo and can be used to infer its age. Although the age of an embryo may be useful for certain analyses, such as interspecific comparisons of growth rates, it must be remembered that age calculated in this manner is at best only an approximation and does not represent primary data obtained from the specimen itself (Velhagen and Savitzky 1998).

TABLE 8

Recommended Standard Data to Be Recorded for Dams[a]

Specimen Number

- Catalogue or field number

Taxonomic Data

- Taxon
- Determined by
- Date determined

Collection Data

- Locality
- Collection date
- Collector
- Associated ecological data

Reproductive Data[a]

- Date of mating (if known)
- Date of oviposition or parturition
- Date of hatching (for oviparous species)
- Holding temperature of dam (if controlled)
- Number of progeny in reproductive event (eggs in clutch or young in brood)
- Specimen number of sire (if known)
- Specimen numbers of progeny

Measurements

- Length (standard measures for taxon)
- Body mass

Preparation Data

- Preparation date
- Preparator
- Euthanizing agent
- Fixative
- Preservative

a. Data repeated for multiple clutches or broods in a breeding colony.

Ethical Considerations

The collection of embryos from the field involves unique ethical considerations. The greatest concern relates to the removal of gravid females or entire clutches from a population. The potential adverse effect of that action on the survival or demographics of a population must be weighed against the benefits of acquiring embryonic material. For most taxa, inci-

TABLE 9
Recommended Standard Data to be Recorded for Embryos

- Specimen Number
  - Catalogue number
- Taxonomic Data
  - Taxon
  - Determined by
  - Date determined
- Parental Data
  - Specimen number of dam (if known; not available for eggs collected in field)
  - Specimen number of sire (known only for captive breeding colony)
- Standard Preparation Data
  - Preparation date (corresponds to date of euthanasia of embryo)
  - Preparator
  - Euthanizing agent
  - Condition at collection (important if embryo died *in ovo* or *in utero* or was damaged during preparation)
  - Fixative
  - Preservative
- Special Preparation Data
  - Histological, whole mount, electron microscopic, or other special preparations (involving all or part of embryo)
- Sex and Measurements
  - Sex of embryo (if known)
  - Length (standard measures for taxon)
  - Egg mass at oviposition (for oviparous species)
  - Body mass at hatching or birth
  - Residual yolk mass (if any)
- Developmental History
  - Mating date of dam (if known)
  - Embryo age (post-mating or post-oviposition, if known)
  - Baseline date (if calculated)
  - Incubation or gestation temperature (incubation temperature of eggs for oviparous species, or holding temperature of dam for viviparous species, if known)

TABLE 9 (*continued*)

- Culture Data (for *in vitro* embryo culture)
  - Date *in vitro* culture began
  - Date *in vitro* culture ended (usually preservation date)
  - Incubation temperature while in culture
  - Culture medium
  - Additional treatment (e.g., use of growth factors or antibiotic-antimycotic solution)
  - Experimental procedures while in culture
- Staging Data
  - Staging sequence (name or citation of staging system employed)
  - Stage number (including substage, if applicable)
  - Staged by
  - Date staged
  - Staging criteria (major morphological features used to assign embryo to stage)
- Illustrations
  - Photographs or drawings of embryo

dental collection of an occasional gravid female is unlikely to exert a critical effect on a population's survival. However, for studies involving more intensive collection of embryos from the wild, investigators should choose their taxa and collecting sites carefully to avoid reducing recruitment or genetic variability.

Collection of embryos expands our knowledge of biodiversity to include early life history stages, in which the process of morphological assembly occurs and during which ontogenetically labile structures may appear. Embryos can clarify homology (Jamniczky and Russell 2008; Fabrezi et al. 2009) and provide additional characters for phylogenetic analysis (Mabee 2000; Werneburg and Sánchez-Villagra 2009). Comparative developmental studies can lead to unique insights into how evolutionary mechanisms function (Rieppel 1993; Valenzuela et al. 2006; Gomez et al. 2008) and how novel structures arise (e.g., Gilbert et al. 2001; Vickaryous and Hall 2008; Vonk et al. 2008). For these reasons, we urge individuals involved in surveys of biodiversity to expend the additional effort required to collect embryonic material whenever possible. Such efforts may involve holding gravid females of oviparous taxa until eggs are laid and then preserving embryos from the clutch on a regular schedule. For gravid females of viviparous taxa, collection of embryos in the field is more difficult. Nonetheless, we recommend that, at the least, gravid females that are collected as vouchers be dissected immediately following euthanasia and that their contained embryos be fixed according to the methods we have described. The systematic collection of reptilian embryos lags far behind that of adults, and a concerted effort to remedy that situation will be required if biologists are to extend their knowledge of reptilian embryonic development beyond that of a few "model" species.

TABLE 10

Publications of Staging Sequences in the Normal Development of Selected Reptiles[a]

Chelonia

Bataguridae: Tan et al. 2001
Carettochelyidae: Beggs et al. 2000
Cheloniidae: Crastz 1982; Miller 1985
Chelydridae: Yntema 1968
Dermochelyidae: Miller 1985
Emydidae: Mahmoud et al. 1973; Greenbaum 2002
Trionychidae: Tokita and Kuratani 2001; Greenbaum and Carr 2002

Crocodylia

Alligatoridae: Ferguson 1985; Hua et al. 2004; Iungman et al. 2008

Rhynchocephalia

Sphenodontidae: Dendy 1899; Moffat 1985

Squamata

Lizards
Agamidae: Muthukkaruppan et al. 1970
Anguidae: Raynaud 1961
Chamaeleonidae: Blanc 1974
Gekkonidae: Werner 1971; Noro et al. 2009
Iguanidae: Lemus 1967; Lemus et al. 1981; Sanger, Losos, and Gibson-Brown 2008
Lacertidae: Dufaure and Hubert 1961; Dhouailly and Saxod 1974
Scincidae: Pasteels 1970
Snakes
Colubridae: Zehr 1962
Elapidae: Jackson 2002
Pythonidae: Boughner et al. 2007
Viperidae: Hubert and Dufaure 1968

[a] More recent works and/or those that provide relatively complete sequences with good illustrations are emphasized. See Billett et al. (1985) for a comprehensive review of the literature on embryonic development and staging in reptiles up to that date.

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Collecting and Preserving Parasites during Reptile Biodiversity Surveys

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Obtaining parasites from reptiles that are collected during biological diversity surveys or inventories is time consuming, and in the past such collections were rarely made (Gardner 1996). However, parasites and other symbionts are integral parts of the ecological characteristics of organisms and provide important information on biodiversity from the population level to the species level. For example, recent biogeographic analyses of the skinks, geckos, and anoles of some Oceanic Pacific Islands (Bertrand and Ineich 1986, 1987, 1989; Goldberg and Bursey 1991, 2000, 2002; Hanley et. al 1995; Goldberg et al. 2005) could not have been accomplished without parallel studies of the parasite/symbiont faunas that they host. The latter studies were possible only because the parasitic and symbiotic organisms had been properly collected and subsequently deposited in recognized museum collections where they were available for study. We strongly recommend that investigators sample the parasites and symbionts of reptiles whenever they are encountered.

Most researchers are reluctant to assign individuals from an often stressed and overworked field crew to process parasites when the specimens and associated data are considered “auxiliary” or “collateral” to the main focus of the study (i.e., reptiles). On the other hand, the most scientifically valid, efficient, and cost-effective method of obtaining data on the parasite fauna of a group such as reptiles is to preserve the parasites when host animals are being collected and processed in the field. Examples of the types of data that can be obtained when a host is collected include *prevalence* of the parasite among individuals within a population (i.e., proportion of individuals with the parasite), *intensity of infection* on individual reptiles (i.e., number of parasites per individual), and distribution of parasites in or on individuals and among populations of reptiles.

Studies of the systematics and ecological characteristics of hosts and their parasites require proper identification of both groups. When the parasites associated with a host are not collected and preserved properly, species-level diagnostic characters (i.e., morphological characters) are usually destroyed. In addition, improper preservation of parasites severely limits, or more likely prevents, studies based on DNA from those specimens.

Proper handling of parasites is also critical for the correct designation of the host individual for a specific sample of parasites, as parasites may accidentally transfer from one host to another at time of collection. Anthropogenic host-transfer, especially difficult to avoid in the field, can occur from as early as when an animal is collected until, and subsequent to, its final deposition in a recognized collection or museum. For example, *ectoparasites* (organisms that live on the external structures of a host organism) that are attached to a host individual may release their holds and fall from the host after it is collected, remaining in a trap or collecting bag long after the host animal has been removed. Those parasites may then transfer to another host of a different species or of the same species but a different demographic group (e.g., sex, age, breeding status); transfer may occur at the same collecting locality or even subsequently at a different one. Anthropogenic host-transfers occur more frequently when investigators place more than one host animals in a single container or place a host in a collecting bag or container that has been used for another host previously, but not properly cleaned.

In this section, we outline methods for collecting parasites that maximize the amount of morphological and molecular information available from each specimen obtained. The procedures are also designed to minimize the likelihood of anthropogenic host-transfers. An excellent, thorough protocol for sampling parasites of reptiles and amphibian has been published on the Web (Goater and Goater 2001), although it focuses mostly on Canadian species of parasites and techniques used there.

Specimen data are often collected in a field laboratory in assembly-line fashion, with different members of the field crew assigned to different tasks. When an individual finishes his or her task, she or he passes the organism to the next person in line for the next step in processing. Generally, one or two persons are assigned to process parasites. We have oriented our presentation toward assembly-line processing. However, individuals working alone can use the same protocols. In our procedures, every part of every animal that is collected is processed in some way. Pritchard and Kruse (1982), Anderson (1965), and Gardner (1996) have provided additional information on collecting techniques for parasites in general. One general rule applying to the collection of both external and internal parasites as well as host tissues is that investigators wear latex or nitrile gloves while sampling and wash their hands thoroughly afterward to avoid potential infection, particularly from pentastomids (Jacobs 1982).

Collecting Ectoparasites and Ectosymbionts

1. The collector places each live host in a new (i.e., *never* used) bag made of thin (1.5–2.0 mil) plastic. She or he also immediately places any dead host removed from a trap or found as road kill in a new plastic bag to prevent loss of ectoparasites and cross-contamination of hosts. Depending on the size of the host, a 200–350-mm bag is best. We recommend that standard cloth snake bags *not* be used, as they often harbor ticks and mites from previous occupants for extended periods unless thoroughly cleaned, which is almost impossible to accomplish in the field. Without such cleaning, investigators must assume that host transfer has occurred.
2. Depending on the study, hosts are either euthanized (see "Euthanasia," under "Preparing Scientific Specimens," earlier in this chapter) or maintained alive for future release. In the latter instance, the investigator will need to subdue the animal or lightly anesthetize it so that it can be examined carefully.
3. After a host is processed (either euthanized or weighed and measured), the researcher examines its ears, eye areas, skin folds, under-scale spaces, mite pockets (skin invaginations where mites concentrate, found in certain families of lizards; see Arnold 1986), and the rest of the body for external parasites, especially leeches, ticks, and mites. If the animal is examined over a sheet of white paper using a lighted 10X hand lens or a dissecting microscope, parasites that fall from the body are more easily located. All parasites encountered are removed and stored appropriately (see below). Live tortoises are particularly difficult to examine because they reflexively draw their legs into their shells when handled, yet the legs must be extended for the investigator to examine all the skin folds where the shell meets the skin (Fig. 27, in Chapter 6). Parasites collected from different parts of the body are placed with a label in individual 1.5-mm vials of 70 percent ethanol (ETOH) for storage. Subsamples can be placed into cryotubes and frozen directly in liquid nitrogen. The investigator repeats the procedure with the next animal until all animals have been processed. Labels should be made of good quality, 100 percent cotton-rag, acid-free material, and all writing is done with permanent India ink (e.g., Higgins Eternal) or disposable, permanent-ink pens. The label includes the host-specimen identification number or the field collection number of the collector. It also indicates the part of the host body from which the specimens were collected and may include a count of the number of ectoparasites collected.
4. The preparator should carefully inspect an animal that has been euthanized for additional (especially small) ectoparasites. Scales with small mites under them tend to stick up and can be detected by holding a lizard or snake at eye-level and observing it from the tail end forward. The investigator lifts each raised scale with jeweler's forceps and a dissecting needle and examines it for 'suspicious objects' using a dissecting microscope at low power (20–40X); he or she then increases the magnification (50–70X) to see if the object has legs. Fly larvae (bot flies or warbles) can also occur subcutaneously, particularly in the cloacal region of turtles. At present, however, larval bot flies cannot be identified to species using morphological characteristics. Collecting live larvae and rearing them to adulthood in an artificial brood chamber is the only way to obtain a reliable identification based on morphology. The larvae can be kept alive in cheesecloth-covered uncapped vials or .45-caliber polystyrene cartridge boxes until they pupate and emerge. Adult flies are preserved in 70 percent ethanol (Gardner 1996).

Parasites and other symbionts can be removed from a host with forceps, a swab, or a needle dipped in ethanol. A leech, tick, or mite that is embedded in the skin of the host should be carefully pried from the skin, rather than scraped, to ensure that mouthparts are included. Small mites that are attached under scales are collected by lifting the scales and carefully teasing out the small arachnid. Parasitic mites often inhabit the oral cavity, lungs, and trachea, as well (Reichenbach-Klinke and Elkan 1965).

The locations on hosts of all ectoparasites collected are recorded in the field notebook of the collector (Fig. 34). The field notebook should contain a record of the numbers of ectoparasites collected from each body region of the host and the type of vial used for storage (to facilitate locating the organisms years later during museum searches). Ectoparasites and other ectosymbionts are preserved and stored in 70 percent ETOH.

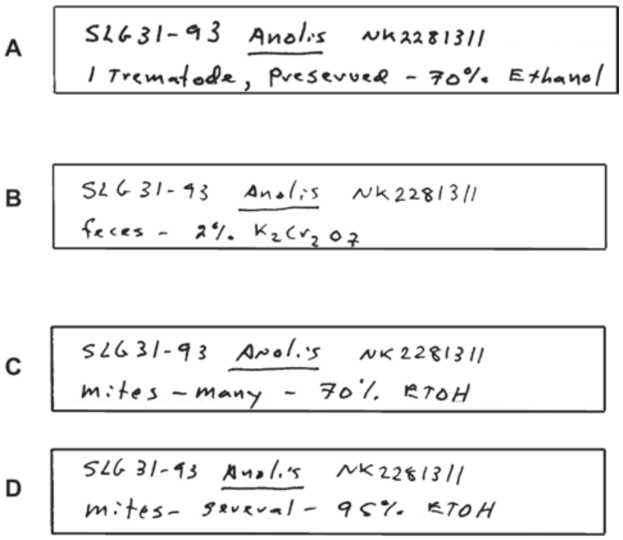

A
SLG31-93 Anolis NK2281311
1 Trematode, Preserved - 70% Ethanol
B
SLG31-93 Anolis NK2281311
feces - 2% K2Cr2O7
C
SLG31-93 Anolis NK2281311
mites - many - 70% ETOH
D
SLG31-93 Anolis NK2281311
mites - several - 95% ETOH

FIGURE 34 Examples of data labels to be placed in vials or plastic bags with parasite specimens. (A) Sample label to be placed in a vial with a single trematode from an *Anolis* lizard. The collector's number (SLG31), the genus of host (*Anolis*), field identification number (93), kind of parasite, and type of preservative in the vial are included on the label. (B) Sample label to be placed in a vial of potassium dichromate with lizard feces from specimen SLG31-93. (C) Sample label to be placed in a vial or plastic bag containing ectoparasites. The collector's number, host genus, field identification number, type of preservative, and general kinds and approximate numbers of parasites in the vial are recorded on the label. (D) Label with the same data as in "C" but also noting that the specimens were preserved in 95 percent ETOH for future molecular and morphological analyses.

Parasites from each individual host must be stored in separate vials.

Live reptiles can be kept for a few hours in wire-mesh cages over pans of water, and ectoparasites that drop through the mesh into the water can be collected with a spoon, forceps, eyedropper, syringe, or filter paper. Ectoparasites can be removed post-mortem by euthanizing a specimen and placing it immediately in an appropriate-size plastic bag with a cotton ball soaked in chloroform or ether. After a few minutes, the bag is shaken to dislodge the parasites and the parasites are recovered and preserved in 70 percent ethanol. The drawbacks of these methods are that the original locations of the dislodged organisms on the host remain unknown and that fumigation does not dislodge all the ectoparasites.

5. After a reptile is removed from a plastic collecting, holding, or kill bag, the preparator collects any ectoparasites that may have fallen from the animal into the bag by squirting a small amount (2–6 ml) of 70 percent ETOH into the bag, washing down the sides so that the alcohol and any bag contents accumulate in a corner. The collector holds the bag so that one corner is over the opening of a small Whirl-pak (plastic bag with integral wire twist-ties), cuts off the corner, allowing the ectoparasites and ethanol to drain into the whirl-pak, and adds a label. This method quickly produces an uncontaminated sample of ectoparasites and ectosymbionts and ensures that no plastic bags will be reused, which is critical for avoiding contamination. Whirl-paks, are small, leakproof, and generally unbreakable—ideal for transporting specimens back to the laboratory, where the contents should be transferred to vials as soon as possible.

Collecting Endoparasites and Endosymbionts

In this section, we discuss only the endoparasites and endosymbionts most commonly collected from reptiles: Protozoa (coccidia), Acanthocephala (thorny- or spiny-headed worms), Cestoda (tapeworms), Trematoda (flukes), Nemata (nematodes), and Pentastomida (tongue worms). Field collectors should keep in mind, however, that novel organisms are sometimes discovered during a dissection.

1. The researcher collects endoparasites from a host specimen after the animal has been euthanized but before other host tissues are removed for genetic analyses. Scissors, forceps, probes, and other implements are always rinsed with 70 percent ethyl alcohol (ethanol, ETOH) and wiped dry with a tissue between animals to avoid cross-contamination with blood or other sources of foreign DNA.
2. Blood smears that are well prepared and of even thickness are important for detecting and documenting protozoa and microfilariae (juvenile filarioid nematodes) that may have infected the reptile being studied. Blood smears can be made from live or freshly dead (i.e., the blood has not yet coagulated) animals. Blood can be collected from any convenient area of a recently dead animal, usually while taking tissues. With a live animal, blood is collected from a toe or tail clip or from the postorbital sinus of most lizards, using a hematocrit tube. Tubes with blood can be sealed with putty and stored. Alternatively, the blood can be blown out onto a slide.

   Typically, an investigator collects blood with a disposable plastic pipette and places a drop (approximately 250 µl) in the middle of a microscope slide. He or she holds another slide at an angle, placing its edge on the first slide in contact the blood drop, and pushes the inclined slide evenly and rapidly away from the blood drop, drawing the blood out into a thin (one cell layer thick) smear (Fig. 35). The field identification number of the host is written on the slide with a diamond-point pencil, and the slide is allowed to air-dry for at least 10 to 30 min, depending on the humidity. New blood smears are fixed at the end of each day (within 24 h of preparation), or sooner if the temperature and humidity are high, by immersing them in 100 percent methanol or 100 percent ethanol for from 2 to 5 min. Slides are stored and transported in standard slide boxes and may be stained and examined after return to the laboratory, or they can be stored and then stained later.
3. To examine the digestive system for endoparasites, an investigator opens the abdominal cavity and severs the esophagus, just above the stomach, and the colon, just anterior to the rectum. Care must be taken not to perforate the organs during this procedure to avoid transfer of parasites from one organ to another, thus invalidating information on the distribution of the parasites within the individual host. The investigator removes the digestive tract intact and places it in a clean plastic or glass Petri dish or beaker with a generous amount of water or saline and a label bearing

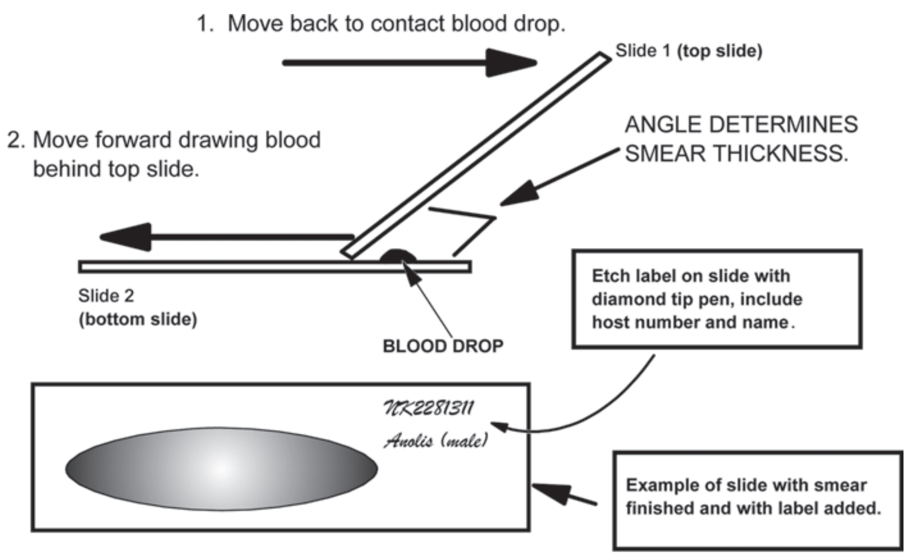

1. Move back to contact blood drop.
Slide 1 (top slide)
ANGLE DETERMINES SMEAR THICKNESS.
2. Move forward drawing blood behind top slide.
Slide 2 (bottom slide)
BLOOD DROP
Etch label on slide with diamond tip pen, include host number and name.
NK2281311
Anolis (male)
Example of slide with smear finished and with label added.

FIGURE 35 Preparation of a blood smear. The smear must be made from fresh, uncoagulated (nonclotted) blood. The angle of the slide above determines the thickness of the smear; a steeper angle creates a thinner smear, whereas a shallow angle provides a thicker smear. Approximately 45 degrees is a good general angle for the top slide. The collector's number (SLG31), host genus (*Anolis*), field identification number (93), kind of parasite, and type of preservative in the vial are included on the label.

the field identification number of the host. If the organs are large, buckets and porcelain pans are used as holding containers. If organs are very large (e.g., from a python, monitor lizard, tortoise, or crocodile), subsamples can be taken from the organs.

4. At this point, the researcher examines the body cavity, liver, kidneys, and lungs for helminth cysts, filarioid worms, and pentastomids. All organs to be removed from the host for future molecular or biochemical-genetic work must be carefully examined for parasites, which are then collected. The presence of parasites in these tissues must be recorded in the field notes that accompany the host. However, because the primers for the host will not pick up the parasites' DNA, the presence of the parasites is not a problem for molecular or biochemical-genetic work. Filarioid nematodes may be encountered in the heart, aorta, pleural cavity, mesenteries, or subcutaneous tissues. Larval or juvenal acanthocephalans, pentastomes, nematodes, cestodes, and trematodes can be found in the liver, mesenteries, or any other tissue. In addition, protistans may be encountered in any of these tissues. Samples should be taken for subsequent investigations for histology and DNA analysis; again, any parasites found should be recorded, even at the most basic level, e.g. "cysts found, protista?" Cysts found should be preserved separately, some of the sample in 10 percent buffered formalin (37% formaldehyde = 100% formalin for the 10% dilution; buffer = 4.0 g mono- [NaH_2PO_4] and 6.5 g anhydrous dibasic [Na_2HPO_4] sodium phosphate/liter 10% formalin) and some in 95 percent ETOH.

   If cestode cysts are encountered, some are fixed *in situ* in the host tissue. The investigator removes a part of the organ with the cyst intact, preserving it in 10 percent buffered formalin. If more than about 10 cestode cysts are encountered in one host, some cysts are removed and carefully cut open. The cestode strobila is placed in distilled water to relax it and then is fixed in 10 percent buffered formalin. If available, some strobili are also stored in 95 percent ETOH, and some are frozen in liquid nitrogen. These various methods of preservation ensure that adequate material will be available for future investigations of both morphological and molecular traits.

5. After examining the body cavity and other host tissues for parasites, the collector frees the intestinal tract from attached mesentery and straightens it, which facilitates using scissors to cut open the intestine along its length. The stomach, small intestine, cecum, and large intestine are placed in separate Petri dishes or other containers, each with a tag bearing the field identification number of the host. Petri dishes must always be cleaned with alcohol or detergent and water and dried between uses. Each organ is opened and examined for parasites. It is important to use scissors with blunt ends, because scissors with sharp points perforate the organ while cutting, making it difficult to open the organ quickly. For small specimens iris scissors are appropriate. An enterotome (special scissors for opening the digestive tract, especially the intestine) is appropriate for opening the intestines of large animals.

   Helminths are often embedded in the mucosal lining of the intestine, which can be removed by scraping the mucosa from the submucosal layer with a microscope slide, tongue depressor, or popsicle stick. If water is abundant, intestinal contents and the intestinal mucosa can be suspended in saline, washed in a fine-mesh soil screen, and then placed in a Petri dish and searched. If water is limited, the intestinal contents are covered with water and gently stirred; the worms sink, and the lighter materials (plant parts and other food items) either float or settle slowly and can be decanted. This procedure is repeated several times, and then the remaining material is searched for helminths with either a dissecting microscope or a 10X jewelers magnifying visor. If helminth specimens are too numerous to count quickly, the intestinal contents should be preserved in 10 percent buffered formalin (with aliquots going into 95% ETOH for DNA work) so that the parasites can be counted accurately in the laboratory.

Organs such as skin, eyes, urinary bladder, trachea, lungs, and gall bladder must also be examined, preferably with a dissecting microscope. Small trematodes commonly occur in the bile ducts and gall bladder of the liver and in the mesenteric veins; small nematodes can be found in the urinary bladder and lungs.

6. Cestodes, trematodes, and acanthocephalans relax and die when placed in distilled water. Tap water or filtered river water can also be used, but *not* saline. Osmotic imbalance causes water to move into the body tissues of the worm leading to osmotic shock and death. The increasing pressure within the body tissues or cavity (depending on the phylum) also may cause the scolex or proboscis to evert. The cestode strobila relaxes when an animal dies from osmotic shock. It is especially important to leave a specimen in water long enough for eversion of the scolex, rostellum, or proboscis, and for relaxation of the strobila or body. Relaxation can take from 10 min to more than an hour, depending on the size of the worm, the ambient temperature, the amount of salt in the water used, and the species. After being relaxed and killed, helminths to be saved for morphological study are fixed in 10 percent buffered formalin and placed in vials with both the field identification number of the host and the location of the parasite in the host; standardized abbreviations (e.g., SI = small intestine, C = cecum) can be used, but a key to abbreviations must be recorded in the field notebook. Parasites from each organ must be preserved separately, in their own vials. Saline is never used to kill cestodes or other platyhelminths, because it prevents osmotic imbalance and subsequent relaxation of the body or strobila.
7. While working with a host, nematodes can be placed in saline temporarily and then transferred directly to a vial filled with 10 percent buffered formalin. Distilled water should be avoided; the osmotic imbalance it produces causes nematodes to burst, and in many cases the specimens are destroyed. Some investigators kill nematodes in nine parts very hot water, brought to volume immediately after immersion with one part formaldehyde to make standard 10 percent buffered formalin. Alternatively, if nematodes are placed in glacial acetic acid (GAA) for a few minutes, they will uncoil and straighten and then can be transferred to and stored in either 10 percent buffered formalin or 70 percent ethanol. Specimens that have been straightened are much easier to identify than those fixed without straightening, because the morphological characters are more readily distinguished. Specimens to be saved for molecular analyses should be washed in saline and then placed in a 1.5-ml nunc (cryo) tube and stored in liquid nitrogen or placed in a vial of 95 percent ethanol. Material being collected for molecular analyses should never be exposed to formalin.
8. If sufficient numbers of helminths are available, investigators should preserve representative individuals in liquid nitrogen or 95 percent ethanol for genetic analyses. Because glacial acetic acid and formalin destroy DNA, individuals preserved for DNA studies must not be treated with either chemical.
9. Investigators should preserve fecal pellets or material from the cecum for later examination for coccidian (protozoa) parasites. Specimens are placed in a vial half filled with 2 percent potassium dichromate ($K_2Cr_2O_7$). A label bearing the field identification number and the generic name of the host is added. We recommend using Wheaton snap-cap vials (15 mg) because one vial half filled with 2.0 percent $K_2Cr_2O_7$ contains sufficient oxygen to keep the coccidia alive for a very long time (morphological structures of coccidian oocysts are visible and can be studied only in living specimens). Recently we found that coccidians of the genus *Eimeria* remained alive after 10 years in 2 percent dichromate stored in a 15-ml snap-cap vial under refrigeration at 2°C (SLG, unpubl. data). The vials rarely leak and can be reused many times.
10. If many conspecific hosts are available, investigators should preserve from two to five entire gastrointestinal tracts individually in 10 percent buffered formalin. This will allow for future examination of the morphological characteristics of the intestines and any associated worms *in situ*. In fact, because of the vagaries of transport of processed specimens from remote field locations, researchers should preserve parasites using many different methods, thus ensuring availability of adequate material for future studies.

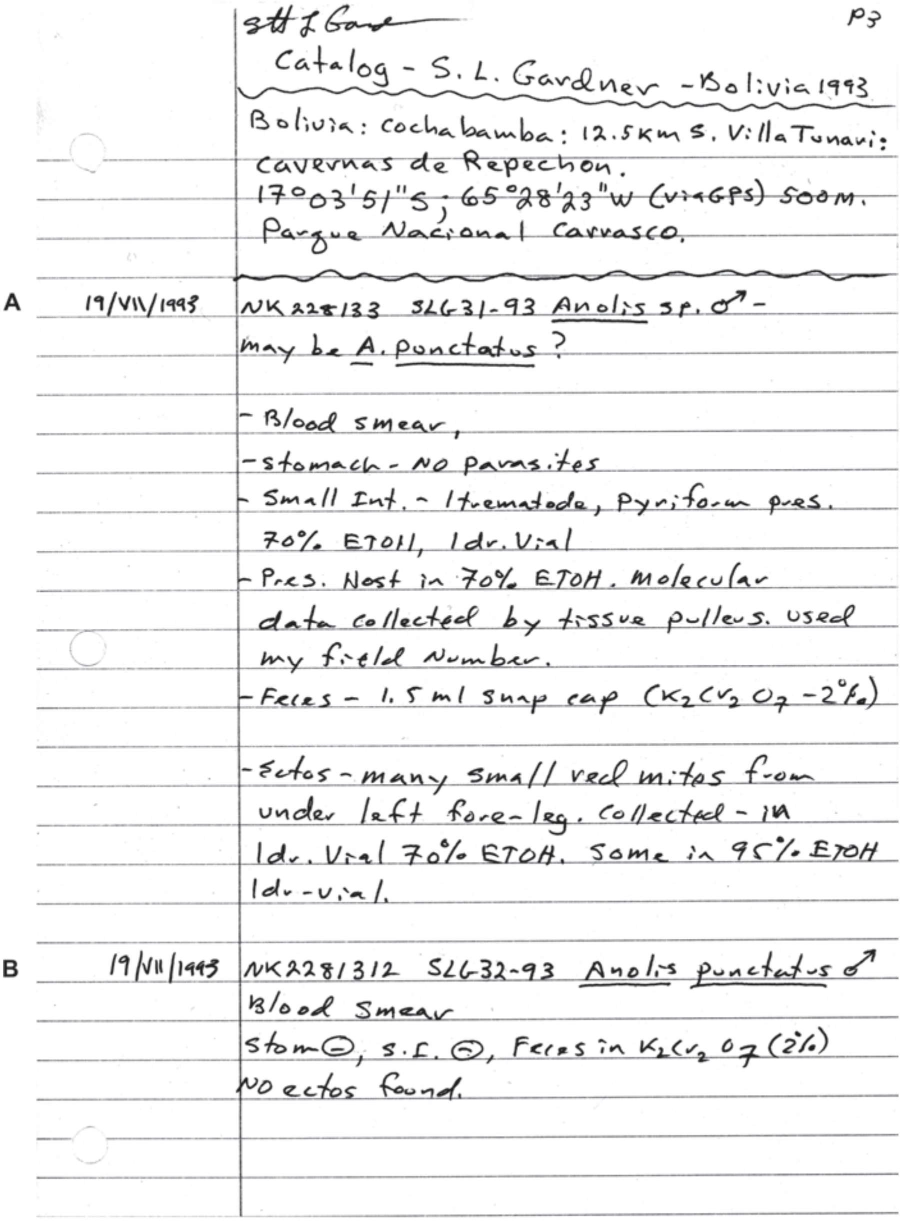
Recording Data

Each researcher should maintain a field notebook (Fig. 36) for recording data on his/her parasitology collections and preparations. The notebook should be made of 100 percent acid-free cotton-rag paper. Only permanent black ink (e.g., Higgins Eternal) should be used to record data. Data can also be recorded in a PDA (see "Handheld Computers for Digital Data Collection," in Chapter 4). However, data stored in an electronic format are much more labile than those recorded in permanent ink on archival paper, and we urge investigators to maintain backup files and printed copies of all data. Collectors should number their specimens sequentially, beginning with 001 and continuing indefinitely, rather than beginning a new sequence each year (e.g., 2008-001, etc.) or each collecting trip (e.g., SLG-001 through SLG-12500).

Each specimen record should include the field collection number of the host to ensure accurate cross-referencing of a parasite sample with the host voucher or symbiotype specimens. A specimen number is always preceded by the collector's initials so that she or he can be identified in the future. In addition, specimen records should include the collecting date and locality, the species name of the host (although acknowledging the provisional nature of field identifications), and the location of the parasite on or in the host. The collector should record the general kinds of parasites encountered in each organ as well as negative searches (Fig. 36).

Materials Needed to Collect Parasites

The materials needed to collect parasites in the field are listed in Table 11. The quantities required will vary with the numbers



~~SL Gard~~ P3

Catalog - S. L. Gardner - Bolivia 1993

Bolivia: Cochabamba: 12.5 Km S. Villa Tunari:
Cavernas de Repechon.
17°03'51"S; 65°28'23"W (via GPS) 500 M.
Parque Nacional Carrasco.

A 19/VII/1993 NK 228133 SLG31-93 *Anolis* sp. ♂ -
may be *A. punctatus* ?

- Blood smear,
- stomach - NO parasites
- Small Int. - 1 trematode, Pyriform pres.
70% ETOH, 1 dr. Vial
- Pres. Host in 70% ETOH. Molecular
data collected by tissue pullers. used
my field Number.
- Feces - 1.5 ml snap cap ($K_2Cr_2O_7$ - 2%)

- Ectos - many small red mites from
under left fore-leg. Collected - in
1 dr. Vial 70% ETOH. Some in 95% ETOH
1 dr.-vial.

B 19/VII/1993 NK 2281312 SLG32-93 *Anolis punctatus* ♂
Blood Smear
Stom ⊖, S.I. ⊖, Feces in $K_2Cr_2O_7$ (2%)
NO ectos found.

FIGURE 36 Sample field notebook entries. (A) Record of examination of *Anolis* SLG31-93 for parasites. Note that the name of the collector and the locality are always included at the top of each page or at any point where the collecting locality has changed. The date is always included with each individual record. The field identification number, followed by the field collector's number is next, followed by the field identification of the host (often, species names assigned in the field are incorrect if new or unknown species are collected). Next, the investigator notes that a blood smear was made; lists the parasites collected from different organs and how they were preserved or stored; records the fate of the host (in this case the whole animal was preserved in 70% ethanol); notes that feces were preserved; and lists the ectoparasites found and how they were preserved. The present entry also indicates the absence of parasites from the stomach. (B) Record of examination of *Anolis* SLG32-93 for parasites. A blood smear was taken, and feces were collected and preserved in potassium dichromate; no other parasites were found. Negative data are always recorded to verify that the host was examined and to ensure accurate estimations of prevalence, intensity of infection, and other ecological parameters.

TABLE II

Materials Required for Collecting Parasites

Materials[a]	*Amount*[b]
Equipment and Glassware	
Dissecting microscope, 0.5–30× magnification	
Magnifying visor, jeweler's 10× or 10× lighted hand lens	
Light source, bright, for working at lab bench; headlamps (e.g., Justrite) requiring 4 size-D batteries work well	
Jeweler's forceps (100 mm), 2 pair	
Blunt-nose gross dissection forceps (120 mm and 140 mm), 1 each	
Copelin staining jar for fixing blood smears	
Porcelain dissection trays, small (30 cm × 20 cm) and large (40 cm × 30 cm)	
Scissors, both sharp-nosed and blunt-nosed of different sizes:	
100-mm iris scissors for fine work	
120-mm blunt-nose scissors for coarse work	
120-mm sharp-nose scissors for cutting tissue	
Rapidograph (or other) pens and indelible India ink (Higgin's Eternal), or disposable Black UNIBALL Deluxe (Faber-Castell) permanent ink pens	
Scalpel and disposable blades (#21) for cutting through host tissue	
Soil sampling sieve, no. 325, USA standard sieve series, 45-µm mesh; 20-cm-diameter small-mesh sieve that catches nematodes but allows colloidal particles in the water to pass through	
Diamond point pen for scribing on glass microscope slides	
Workshop light, fluorescent, for detailed microscope work or work with a magnifying visor	
Liquid nitrogen tank with a static holding capacity of 2 weeks (or sufficient for the length of the collecting trip)	
Squirt bottles of several sizes for the various solutions	
Expendable Supplies	
Plastic bags, 1.5–2.0 mil, rectangular (200 mm × 350 mm)	200
Whirl-pak or other plastic bags with twist tie closures	400
Latex and nitrile gloves	
Microscope slides, standard pre-cleaned (not frosted)	2 boxes
Tongue depressors or popsicle sticks for scraping intestinal mucosa	
Petri dishes, plastic, small, medium, and large	
Insect-pin probes, small	
Dissection probes and needles	
Plastic pipettes, many disposable	100
Field notebook paper, 100% cotton rag, acid free	200 sheets
Vials, Wheaton snap cap, 15-ml	1 box of 144
Vials, Wheaton snap cap, 20-ml	1 box of 144
Vials, screw-cap with Teflon inserts in cap, 1-dram	1 box of 144
Vials, screw-cap with Teflon inserts in cap, 15-ml	
Cryotubes (nunc), with colored caps (or lid inserts); brown tops for parasites	100
Labels (or label paper) for wet preparations inside vials; 100% rag notebook paper or archival (museum)-quality label stock	
Cheesecloth	
Tissues or Kimwipes for cleaning equipment	10 boxes
Paper towels	2 rolls

Materials[a]	*Amount*[b]
Reagents	
Methanol (MEOH), 100%	500 ml
Ethanol (ETOH), 95%; (can be used to make 70% ETOH)	500 ml
Ethanol (ETOH), 70%	1000 ml
Formalin, 100% (= 37% Formaldehyde)	500 ml
Buffering salts (monobasic sodium phosphate, NaH_2PO_4,), either anhydrous, premeasured in 4-g packets, or in a water solution premeasured	5 packets
Buffering salts (dibasic sodium phosphate, NaH_2PO_4), either anhydrous, premeasured 6.5-g packets, or in a water solution pre-measured	5 packets
Potassium dichromate ($K_2Cr_2O_7$), 2% solution	500 ml
Glacial acetic acid	

a. See Appendix II for a list of vendors.

b. Estimated quantities of some disposable supplies needed for collecting parasites from approximately 100 reptiles.

of collectors, the duration of the fieldwork, and the number of animals collected. We have estimated the quantities of various disposable supplies listed in the table needed for collecting parasites from approximately 100 reptiles. For general collecting, researchers will need approximately one large plastic bag, two Whirl-pak bags, two 1-dram and two 15-ml snap-cap vials per host animal. Actual use will probably be lower, because not all hosts are infected with parasites.

Acknowledgments

We thank Agustin Jimenez-Ruiz, Robert L. Rausch, Robert M. Storm, and Donald W. Duszynski for long-term support and encouragement during the development and implementation of the techniques we outline.

Collecting and Preserving Tissues for Biochemical Analyses

James A. Schulte II

Tissue samples for biochemical analysis are an increasingly important resource for many biological studies. They can be used in biological investigations in a broad range of disciplines, including population biology, ecology, biogeography, systematics, conservation, evolution, environmental toxicology, and genomics.

Several methods can be used to collect tissues with viable DNA from specimens acquired in the field. Snap- or quick-frozen tissue samples, however, have the greatest current and future utility. They can be used for sequencing DNA and microsatellite markers as can material obtained from large DNA-insert libraries, such as BACs (bacterial artificial chromosomes), particularly when projects require relatively large amounts of intact DNA with little degradation. I recommend that, whenever possible, tissue samples be taken, snap frozen, and stored. I will discuss other techniques that can be used if snap freezing is not possible, as well as their limitations

There is a vast literature on issues related to sampling tissues for biochemical analyses. Numerous references and additional details regarding the methods presented below are provided in Dessauer and Hafner (1984), Hillis et al. (1996), Duellman (1999), Prendini et al. (2002), and Simmons (2002). However, techniques for preservation and storage of tissues and maintenance of the large collections of frozen tissues found in many museums are constantly being tested and improved. Several publication venues (e.g., *Molecular Ecology Notes, Biotechniques,* and *Herpetological Review*) in which researchers experimenting with techniques can share modifications and improvements with colleagues are available.

Vouchers for Tissue From Animals in the Field

The importance of depositing a well-documented voucher specimen in a major collection for association with a tissue sample cannot be overstated (see Chapter 6, “Voucher Specimins”). Proper identification and documentation of organisms from which tissues are obtained is essential for maximum utility of the sample. In fact, future verification and repetition of a molecular study is impossible unless tissues used are accompanied by vouchers and their associated data. One of the benefits of molecular data is the ability to recognize distinct species previously unnoticed by even the most knowledgeable systematists. From a biodiversity perspective, we may be farther behind than originally perceived in our documentation of the earth’s biota, because of large numbers of still-undiscovered cryptic species. Without voucher specimens to compare with previous collected material, preparing accurate, detailed species descriptions is extremely difficult.

Sample Size

The number of samples required for a study depends on the primary focus of the investigation. For detailed population-level studies, a large number of individuals (>20) must be sampled so that intra- and interpopulation variation can be evaluated. The population structure of a species is influenced significantly by a variety of factors such as body size, home range size, population density, and taxonomic group. The population structure of a large terrestrial snake, for example, will be radically different from that of a small arboreal skink. A preliminary evaluation of the target organism's natural history and geographic distribution will greatly improve the ability of the investigator to determine the number of samples required.

On the other hand, a single individual is better than none. For interspecific studies, especially of those groups with large numbers of species, an individual from as close as possible to the type locality is ideal. It is becomingly increasingly evident that species or species complexes with large geographic distributions are composed of several distinct lineages, so a single individual is not likely to represent the species adequately. By sampling as close as possible to the type locality, an investigator can minimize confusion regarding the species represented by a particular gene sequence. Therefore, it is essential to report detailed locality and voucher specimen information for each published DNA sequence or fragment mentioned in a manuscript. Those data also must be associated with the annotated gene sequence deposited in molecular sequence databases (see Appendix II) such as those maintained by the European Molecular Biology Laboratory (EMBL), GenBank, or the DNA Data Bank of Japan (DDBJ).

Specimen Documentation

DATA

Documentation of specimen information should be as complete as possible and written with water- and solvent-resistant ink in an equally resistant notebook. The following data should be recorded in the field notebook for each tissue sample: date, time, collector, locality with latitude and longitude, field number of tissues and associated voucher, species identification, sex, mass, type(s) of tissue taken, storage method (i.e., snap frozen, 95% ethanol, buffer type), brief descriptions of the voucher (e.g., color or abnormalities) and habitat, and other miscellaneous information that may be useful such as notes on ecology or behavior.

LABELS

Tissue specimens should be labeled with the collector's initials and the field number that corresponds with the voucher specimen. If tissues are stored in ethanol or buffer, or frozen, the information is written in pencil or permanent, cryoproof ink (India or Rotring) on a small piece of sturdy cotton-stock paper or a polyethylene (Kimdura, Tyvek, or Polypaper) tag and placed in the cryotube with the tissue. The investigator should test the writing media before the field trip to make sure that the pencil will not rub off or the ink will not ink run in the ethanol. She or he should also label the outside of the tube with the species identification, collector's initials and field number, and tissue type, using permanent ink, a diamond-tipped pen, or a heavy lead pencil. This information facilitates storage and retrieval of cryotubes after they have been accessioned into a collection.

Permits

Obtaining proper documentation to collect tissue and voucher specimens is essential. Museums require copies of all relevant forms and permits before they will accession specimens into their collections. Most countries now have strict laws to protect their biodiversity and genetic resources, and obtaining necessary permits often requires several months. Special regulations often apply to collection of threatened and endangered species and species of special concern. Researchers should familiarize themselves with all state, federal, and international regulations for collecting, transporting, and importing samples for biochemical analyses and their associated voucher specimens.

International regulations concerning sampling of genetic material are often cumbersome. At least 6 months is commonly required for preparation of applications and final approval. Many countries require permission at the local, state, and federal levels, as well as from multiple agencies. For example, a country may require an investigator to obtain written (preferable) or oral permission from some or all of the following agencies: Fish and Wildlife Service, Department of Fish and Game, Department of Natural Resources, State or National Park Service, Department of Agriculture, Department of the Environment, and land owners. The researcher should document contact information each time any contact is made with an agency. Also, a permit granting permission to collect whole specimens should state explicitly (not to be assumed) that permission to remove tissue samples from the study organism is also being granted. Investigators should also be aware that most countries require a permit to export specimens, in addition to the collecting permit. Working with a collaborator in the country of interest is an invaluable addition to any study from both a logistics standpoint and for general exchange of ideas and scientific information.

In the absence of a special waiver-granting permit, all tissue and other specimens must be imported into the United States at one of 14 designated ports of entry: Atlanta, Baltimore, Boston, Chicago, Dallas/Ft. Worth, Honolulu, Los Angeles, Miami, New Orleans, New York, Newark, Portland, San Francisco, or Seattle. The person transporting the specimens must declare them on the Customs Declaration Form and have a completed U.S. Fish and Wildlife Service (USFWS) declaration document 3-177, as well as other relevant specimen documentation (e.g., species list, collecting and export permits from the country of origin, or museum loan information) available for inspection by the customs officer. Advance notification of the USFWS at the port of entry will expedite this process.

Collecting specimens in the United States is no less regulated. Depending on the scope, location, and duration of the project, an investigator may be required to obtain permits from numerous agencies. A good place to start is with the state Fish and Game Agency or Department of Natural Resources. In addition to species federally protected under CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and Endangered Species Act regulations, each state has its own list of species that it considers endangered, threatened, or of special concern. States issue special permits for work on these organisms. Information regarding state and federal permits and protected species is generally available on agency websites. Detailed specimen information for all material collected is obligatory in the final report at the termination of the permit term.

Packing and Shipping

Liquid nitrogen tanks, also called Dewar flasks or refrigerators, are used to freeze and store tissues, most often for weeks or months. These flasks come in various sizes at a range of prices.

Liquid nitrogen is available in larger cities but may be difficult to obtain in smaller, remote places. Current airline regulations strictly control the transport of free liquid nitrogen, making air transport of traditional liquid nitrogen tanks impractical (ground transport is less prohibitive but takes more time).

I recommend that dry shippers be used according to manufacturers' specifications to transport snap frozen specimens. Dry shippers will keep material frozen in the field for up to 3 weeks, are International Air Transport Association compliant (IATA 2010), and do not require free liquid nitrogen for transport. Airlines typically require passengers transporting dry shippers to contact their Hazardous Materials Department prior to departure. Tissues can also be frozen and stored in the field in dry ice, which may be more readily available than liquid nitrogen. Air transport of dry ice is permitted under strict regulations. Investigators should consult the most recent IATA manual (see Appendix II) for regulations governing the shipping of dry ice, with particular attention to the dry-ice acceptance checklist. Most airlines will accept no more than 2 kg of dry ice in a properly labeled and sealed container.

Self-contained frozen cold packs (Blue Ice or Techni-Ice) in insulated shipping boxes can keep specimens frozen for a few days and have been used for air transport. Packages should be leakproof. Ethyl alcohol and isopropanol are classified as class-3 hazardous materials, and up to 500 ml may be shipped as air cargo. Volumes greater than 500 ml must be packaged, labeled with a HazMat form, shipped according to IATA regulations (IATA 2010), and declared as dangerous goods (class-3 flammable liquid, packing group 2).

Sampling and Preserving Tissues in the Field

Tissue sampling equipment required in the field is listed in Table 12. Initial preparation of materials before tissues are removed from the animal to be sampled will significantly reduce processing time if a large number of tissue samples are to be collected. The investigator should (1) record detailed specimen and sampling information in his/her field notebook, (2) add linen or cotton strings to specimen tags (this can also be done before going into the field), (3) write the specimen field numbers on small pieces of sturdy cotton paper or polyethylene tags and place them in cryotubes (Nalgene or Nunc tubes), (4) label the tubes, and (5) prepare a fixing tray for processing voucher specimens. At least two sizes (2 ml and 5 ml) of self-standing cryotubes should be available; an internal thread and silicone gasket offers the best seal. Other materials that can be useful are cardboard or plastic boxes for organizing and storing cryotubes, a small rack in which cryotubes can stand upright, and a box or two of Kimwipes. Forceps and scissors must be thoroughly cleaned (see below) before each tissue is extracted, and tissues should be removed under conditions that are as cool as possible.

TABLE 12

Equipment Required When Sampling Tissues for Biochemical Analysis[a]

Buffer solution (e.g., RNAlater, Longmire Buffer, 20% DMSO, etc.)
Catalog
Chloroform
Cryotubes (2 and 5 ml)
Cryomarker
Cryotube rack
Diamond-tip pen
Ethanol (at least 95% without additives)
Forceps (at least 2 pair)
Freezing material (liquid nitrogen or dry ice)
Headlamp
Kimwipes
Matches
Laboratory gloves
Lighter
Linen (cotton) string
Liquid nitrogen tank
Nalgene bottles (2–3 small, ~30ml to aliquot ethanol or buffer)
Nembutal (Sodium pentabarbitol)
Orajel
Paper towels
Pencil (and sharpener)
Pens (archival ink)
Scalpel
Scalpel blades
Scissors (fine tip, at least two pair)
Soap with high ammonia content (e.g., Micro)
Specimen tags
Styrofoam ice chest
Syringes (various volumes, 1 ml to 20 ml)
Syringe needles (various gauges)
Tongs

[a] See Appendix II for a list of vendors.

After the materials are prepared, the investigator kills the animal to be sampled with an anesthetic or by freezing (see AVMA Guidelines on Euthanasia June 2007). Once body functions have ceased, the investigator makes a small slit in the body wall through which to extract the organs from the body cavity. Organs that can be extracted include the heart, spleen, leg or arm muscles, stomach and intestines cleaned of all debris, and liver, from which the gall bladder must be carefully removed to prevent bile from interfering with DNA extractions and inhibiting downstream reactions. The organs of each individual are placed in a single cryotube (or they can be placed in separate tubes with lids color-coded for each tissue type). Adequate space should be left at the top of the tube for expansion of frozen tissue. The tube is immediately placed in liquid nitrogen or dry ice to snap freeze the tissues. If the tissues are preserved in ethanol, the liquid-to-tissue ratio should be at least 3:1 to maximize tissue desiccation rate. After 1 to 2 hours or upon return from the field, the liquid in the tubes is replaced with fresh ethanol of the same concentration for long-term storage.

The tag with the corresponding specimen number is tied to the voucher specimen, which is prepared in the fixing tray. Freezing whole specimens is not recommended, as they do not fix well once frozen.

Scissors and forceps should be cleaned thoroughly after the desired tissue(s) are extracted from an animal. This is essential, as contamination is a common problem especially when multiple individuals are sampled from a single population. DNA extractions and polymerase chain reactions are extremely sensitive, and only a few cells are sufficient to contaminate a sample. If adequate water is available, investigators should use high-ammonia-content soap (e.g., "Micro") to remove all blood and tissue from their tools and then rinse them thoroughly. If clean water is not readily available, ethanol-soaked Kimwipes can be used to clean the tools of all blood and tissue. Tools can also be heat-sterilized using a lighter or matches. However, DNA is quite heat resistant, so this method may be less reliable.

Nonlethal Techniques for Sampling and Preserving Tissues

Materials differ greatly in their utility as a source of viable DNA and DNA yield. External tissues, such as toes and tail tips, generally work well for most DNA sequencing and fragment data if enough soft tissue is taken with the sample. Muscle plugs and whole blood work well in most applications, including DNA sequencing and fragment analyses. Muscle plugs obtained using a steel cork borer should be snap frozen or stored in one of various buffer solutions or 95 percent ethanol for subsequent storage in a –80°C freezer. Whole blood is removed using a syringe or capillary tube and stored in buffer solutions in cryotubes (see below). Several drops of blood removed from a limb or tail base can be stored either on commercially available specially buffered Guthrie cards, Whatman FTA cards, or on common laboratory Whatman paper. The cards or paper are light and easily stored in ziplock bags so that large numbers of samples take up minimal space. Silica-gel beads can be included in the bags to facilitate desiccation. Recent experiments in my laboratory have successfully extracted high-quality DNA form FTA card punches following the blood protocol in Qiagen's DNeasy Blood and Tissue Kit.

Other materials that yield adequate amounts of DNA for many common molecular applications include mouth scrapings, egg-shell membranes, and shed skins (Bricker et al. 1996; Clark 1998). Viable DNA has also been obtained from scute clips of freshwater turtle hatchlings (Mockford et al. 1999). However, extracting DNA from external tissues such as scales, claws, fecal matter, and secretions (e.g., from femoral pores) is difficult, and the viable DNA may be highly fragmented.

Preservation

CHEMICAL

The factors most commonly responsible for DNA degradation and its reduced utility are exposure of tissues to water and/or heat. If it is not possible to freeze tissue samples, they can be maintained in at least 95 percent ethanol or certain buffers for an undetermined period. Note that some countries put additives into ethanol to make them unsuitable for human consumption. Some additives significantly reduce DNA quality, so additive-free ethanol is preferable. RNAlater (Ambion) is rapidly becoming popular for tissue preservation because it will preserve both RNA and DNA, with genetic material remaining viable at room temperature for a few days without freezing. Lysis buffers provided with DNA/RNA extraction kits or purchased separately, from companies such as Qiagen or Promega, also have been used successfully to store tissue. Any one of several commonly used buffers (e.g., 0.25 M EDTA; 2.5% SDS; 0.5 M Tris-HCl, pH 9.2) or a salt (NaCl)-saturated DMSO (dimethylsulfoxide) solution may be the more cost-effective options. Silica beads are commonly used to preserve mammalian scat samples. Buffers used for storing blood include Longmire buffer (Longmire et al. 1988), 2 percent 2-phenoxyethanol with glycerol or DMSO added as a cryoprotectant, or 2-propanol with EDTA or SDS for cryoprotection (Prendini et al. 2002). As a last resort, viable DNA has been obtained from samples stored in laundry detergent if none of the previous solutions is available (Kuch et al. 1999). The reported long-term effects of these latter fluids on tissues and the subsequent utility of such tissues are variable and require additional investigation.

CRYOGENIC

Tissues should be stored in a –80°C freezer or a –150°C liquid nitrogen tank whenever possible, and the number of freeze-thaw cycles to which each specimen is exposed should be minimized. A small drop of glycerol or DMSO buffer can be added to a tissue sample before preservation in the freezer to minimize tissue damage from freezing.

Preparing Samples for Histological or Chemical Analyses

Research into the decline of amphibian populations has reinforced the importance of evaluating developmental anomalies resulting from exposure to a toxin or pathogen in various organisms. Many toxicological assays can be performed using frozen tissues that have been stored at –80°C. Cell damage occurs when a tissue is subjected to freezing and thawing in liquid nitrogen; damage can be reduced with the addition of glycerol or DMSO, which act as cryoprotectants. Some fine-tuning of the amount or type of cryoprotectant with tissue volume and type may be necessary to minimize damage to the cells. Most proteins used for isozyme electrophoresis studies are highly stable. Snap-frozen tissues should be adequate for these studies, although the amount of starting material required may be greater than that required for a standard DNA sequencing analysis. Frozen samples are of more limited use in histological analyses than those preserved in alcohol or buffers, as soft tissues, organelles, and cells are often destroyed during the freezing process. A small piece of tissue should be fixed in preservative such as 10 percent neutral buffered formalin without freezing for histological examinations. Formalin is acidic and should be buffered to a pH of approximately 7.0 (Nagorsen and Peterson 1980; see "Fixatives" under "Preparing Scientific Specimens," earlier in this chapter).

Whole blood from reptiles can be obtained by heart puncture (sedation often required) using a sterilized syringe, or from the orbital sinuses using glass microhematocrit tubes. It can also be taken from the cervical sinus of turtles and caudal vessels of lizards and snakes with a syringe. Plasma should be removed from the blood with an electric or hand-powered centrifuge before it is frozen. Blood can also be stored in various buffers, including 2 percent 2-phenoxyethanol with glycerol or DMSO added as cryoprotectants, or 2-propanol with EDTA or SDS for cryoprotection (Prendini et al. 2002). One millimole of EDTA or 1 milligram of heparin can be added to 100 ml of blood to prevent clotting (Dessauer et al. 1996). Hematocrit tubes are wrapped in corrugated cardboard to prevent breakage during transport.

Karyotyping

A *karyotype* is a preparation of the chromosomes in a eucaryotic cell that displays their number and morphology. It also refers to the set of chromosomes of an individual or species. Karyotypes have been used frequently in phylogenetic studies, as well as for the identification of chromosomal abnormalities and cytogenetic mutagenesis resulting from environmental perturbations. Sessions (1996) outlined five steps in preparing standard karyotype slides of mitotic metaphase chromosomes: (1) obtain tissues with high mitotic activity, (2) expose them to a mitotic-arresting agent, (3) treat the cells or tissues with hypotonic saline, (4) fix and store them, and (5) permanently mount the chromosome preparation on slides. Genetic material derived from steps (1) to (3) also can be used to extract material for chromosome banding, *in situ* hybridization, chromosome painting, and DNA sequence extractions (see Sessions 1996). Kits for making chromosomes preparations are also available, but they increase costs.

Mitotic chromosomes can be obtained from a variety of tissues, including kidney, spleen, intestinal epithelium, bone marrow, and testes. About 0.1 ml/g body weight of a 1.0 to 5.0 percent aqueous solution of colchicine is injected into a healthy animal and left to incubate for approximately 4 hours for an average-size small reptile such as a North American Gartersnake (*Thamnophis* spp.) or Spiny Lizard (*Sceloporus* spp.); more incubation time is required for larger species and turtles. The animal is anesthetized (see "Anesthesia in Reptiles," in Chapter 8, and AVMA Guidelines on Euthanasia June 2007), and the spleen, kidney, and gonads, as well as the stomach and intestines, are opened lengthwise, cleaned, and removed. Small incisions are made in the tissues, which are placed in approximately 50 ml of clean distilled water in a flask or beaker and stirred constantly. If the water becomes dirty, it should be replaced with fresh water. After 10 to 15 min, the tissues are removed, blotted dry with a paper towel, and immersed in 50 to 100 ml of a freshly prepared, ice-cold fixative solution of 3:1 ethanol and glacial acetic acid for 15 min on ice. The fixed tissues are transferred with fresh, cold fixative to either a glass scintillation vial or a large plastic cryotube suitable for storage at ultracold temperatures, and stored at –20°C or colder. The fixative can be used for additional specimens if kept cold and if contamination with water is minimal, especially in humid climates. Fixation quality may be negatively affected if the concentration of fixative is incorrect.

A test slide labeled with the field number of the appropriate voucher specimen should be prepared and the quality of the chromosome preparation assessed. A clean, ice-cold, wet slide is shaken over paper towels and then held at a 30° angle over the towels while several drops of the tissue suspension are splashed onto the slide from about 0.5 m above. Blowing gently on the surface of the slide and then igniting the fixative mixture with a match or lighter will dry it. Cell density can be adjusted as necessary by diluting the tissue suspension or by resuspending the cells in a smaller volume of cold fixative. Cells should be spread evenly on the slide so that they are not in contact.

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CHAPTER EIGHT

Dealing with Live Reptiles

MERCEDES S. FOSTER
Chapter Coordinator

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Ethical Considerations in Working with Reptiles

Gordon M. Burghardt

In recent years there has been a major reconsideration of how animals are treated during field and captive research as well as instruction sponsored by zoos, educational institutions, and government agencies. Many countries have placed major limitations on research methods used in both captive and field settings; these restrictions also apply to animals that are "collected" (i.e., killed/preserved) upon capture in the field. Most U.S. institutions, public and private, have had to implement review procedures for use of vertebrate animals, often including formal establishment of IACUCs (Institutional Animal Care and Use Committees) with involvement of veterinarians, lay members, and scientists. Many of these committees are now major bureaucratic entities with considerable authority in approving, funding, modifying, challenging, delaying, or otherwise influencing the course of research involving any vertebrate species. It is often important, if not mandatory, to obtain approval for research methods even before applying for research grants. This is true even if controversies over which animals are included in the Animal Welfare Act and which species need to have their psychological well-being addressed are ongoing, and to date do not apply to birds, rodents, or animals used in agricultural research, let alone nonavian reptiles. Regardless, a proactive stance is warranted even when regulations do not apply, as the accepted practices of today may be deemed unacceptable in the near future and may compromise, or even invalidate, the application of current research findings in the future.

The primary goal of this section is to raise questions for which we need answers, as well as to sensitize field researchers to the need to carefully reassess many common methods that may be ethically compromised and in need of modification, replacement, or the development and articulation of revised or new rationales to justify continuing their use. This is especially true if more effective methods exist, many of which are described in this volume. Essentially, researchers and their field assistants need to be committed to ethical treatment of the animals they have been entrusted with studying. It is not sufficient just to satisfy committees and review panels. If herpetologists are indeed moved by the mysteries of nature and the importance of nonavian reptiles in the natural world (e.g., Greene 1997), then scientists should be leaders in educating the public and should show their respect by example. Given the endangered and threatened status of many reptiles and the low repute in which many people hold them (except most turtles and many lizards), this may be particularly relevant in their case. Recent publicized fears, some justified, of introduced squamates such as Burmese Pythons (*Python molurus bivittatus*) and Nile Monitors (*Varanus niloticus*) in Florida and the conservation problems posed by even seemingly benign Red-Eared Sliders (*Trachemys scripta elegans*) create additional challenges that merit nuanced responses and evaluation.

Philosophical Issues

It is ironic that the major intellectual pressure for altering our treatment of animals in research stemmed from philosophers. These academics often had limited biological knowledge but were more willing than most evolutionary scientists to consider seriously the implications of phylogenetic continuity for issues of animal use and animal welfare. This has now led to an enormous literature, with scientists often themselves studying the many ways in which we may be mistreating animals or failing to provide them with proper conditions in captivity or during the research enterprise. Specialist journals

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Reptile Biodiversity: Standard Methods for Inventory and Monitoring, edited by Roy W. McDiarmid, Mercedes S. Foster, Craig Guyer, J. Whitfield Gibbons, and Neil Chernoff. Copyright © 2012 by The Regents of the University of California. All rights of reproduction in any form reserved.

(e.g., *Journal of Applied Animal Welfare Science*) have even been established, and these publish papers that include reptiles.

I cannot present all of the various current approaches and controversies in this area today, but most lead to a similar critique of the research enterprise and focus on the three "Rs": Refinement, Reduction, and Replacement (Russell and Burch 1959). For behavioral, ecological, and conservation studies with reptiles, the last R is not possible, except for substituting a more common species or subspecies (e.g., Valley Gartersnake, *Thamnophis sirtalis fitchi*) closely related to a rare or endangered one (e.g., San Francisco Gartersnake, *T. s. tetrataenia*) that may be the actual target taxon. Certainly, however, refining our methods to reduce impacts on wildlife and habitats and reducing the number of animals captured or killed may often be possible. If control of an invasive introduced reptile is necessary, the problems are different, but even in such situations humane control methods should be the goal. The recent book by Morrison (2009), a staunch critic of the animal rights movement who did much invasive research on animals and was intimately involved in many of the controversies framing the development of policies on use of animals in research, is a revealing and honest account of how his perspective has changed while still supporting animal research. It also provides a review of ethical and policy debates by an "insider."

Typically, philosophical issues about treatment of animals in research have fallen into two camps: The *utilitarian (cost-benefit) approach* popularized by Singer (1975) and the *deontological (rights-based) approach* of Regan (1983). The former is a concern with the costs and benefits of the intervention (with costs weighted toward animal suffering), whereas the latter is based on core principles or rights (rights to a life) that may trump benefits to animals other than those used in the study, even if conspecifics! Thus, veterinary research on dogs to develop improved methods of dealing with disease or injury would not be viewed as ethical unless it also aided the subject dogs, and it certainly would be condemned if the study involved nonsurvival surgery, for example. If not justifiable with people, then other species should be off the table also. This deontological approach has often led to the same conclusions, in practice, as the utilitarian approach. As Singer argued, since most experiments are failures or do not lead directly to significant benefits to humans or other species, then all the sacrificed animals need to be considered in the utilitarian cost-benefit calculation. These two approaches framed the early controversies on animal research (Russo 1990), and an understanding of some of this history should be part of the training of all biologists, as it continues to have an impact on debates over regulations and how they are implemented. While these extreme positions are not reflected in current research regulations, and most members of animal care and use committees take a more balanced approach, the fact remains that almost everyone has some limit beyond which research methods are unacceptable, and the bar is being raised continuously as we find out more about the cognitive and affective lives of nonhuman animals, reptiles included.

Russo (1990) provided a good, compact introduction to these approaches to laboratory animal welfare, but much more has been written on the topic (e.g., Bekoff and Meaney 1998). Russo was also one of the first to try to compare and integrate these approaches with a more ecological or holistic concept of animals and their role in the world. The "land ethic" view of Leopold (1987), on the other hand, has greatly influenced the modern environmental movement, along with many other conservation-oriented thinkers (Callicott and Frodeman 2008). Conflicts between those who focus on the welfare of individual animals and those concerned about ecological integrity have still not been resolved. Researchers should be aware that for most IACUCs, claims that questionable procedures are justified because they may have laudable conservation benefits or provide useful general principles will not excuse insensitive procedures, even if they are less expensive than an alternative. The guidelines approved by the Animal Behavior Society and the Association for the Study of Animal Behaviour (2002) are useful for initial guidance.

Applications to Reptiles

Researchers need to recognize from the earliest stages of planning a study that almost everything done with live reptiles (and all other animals) has some ethical dimension. Even the most benign procedures may have unintended consequences, and the reach of this conclusion will only expand. Just observing an animal in the field, even for a short time, let alone capturing and marking it or keeping it in captivity, may be ethically suspect for many reasons. Do the procedures have short-term (pain, stress) or long-term detrimental effects on the individual? Alterations in behavior due to observer presence, trapping methods, provisioning of a population with extra food, or modifying habitat may alter individual behavior, social dynamics, and nontarget organisms. Adverse consequences for the population (or even the species) are possible, especially when destructive sampling for collections or research study is undertaken. The effects of research procedures on population viability is an essential aspect of research carried out for purposes of measuring and monitoring biological diversity, but one not often explored by IACUCs, which focus on individual treatment. However, research methods that involve manipulation, risk of disease transmission, injury, exploitation of particularly vulnerable demographic elements of a population (e.g., large females in many snakes, nesting aquatic turtles, roaming large male iguanas), and other consequences for the studied population are legitimate concerns.

A problem for those working with nonavian reptiles is that the experience (let alone expertise) of IACUC members with ectothermic species is often limited. Central animal facilities established for housing animals have often been designed without the requirements of reptiles being taken into account (e.g., temperature, humidity, diet, space, cage structure, etc.). Furthermore, because most committee members lack experience with field research, it is often difficult for them to evaluate field methods for capturing, handling, marking, transporting, anesthetizing, euthanizing, and implanting animals in field settings. It is important for such committees to have the requisite expertise to make judgments based on sound science. IACUC members may greatly appreciate appropriate sections of this volume and other sources as references. IACUCs are concerned with facilitating research, but also they often are legitimately concerned about loss of institutional accreditation, bad publicity, and research methods that, if found to be flawed, could jeopardize other ongoing research at the institution, as well as external funding. For that reason, protocols submitted for reptile research should provide ample description of the methods to be employed and outline how standards of feeding frequency, temperature, humidity, handling, and euthanasia for mammals may not be appropriate. It is especially important to justify naturalistic environments that provide ecologically relevant features involving structures, substrates, retreats, and so forth. The American Veterinary Medical Association (AVMA) has been proactive in developing guidelines, such as for euthanasia, and using them in protocols can save time and avoid unnecessary conflict with veterinarians and IACUCs. Environmental enrichment is now mandated in many situations, and in the future, researchers with reptiles may have to justify the rather sterile conditions in which most research reptiles are kept. Studies showing the importance of enrichment with reptiles are now appearing with greater frequency (e.g., Almli and Burghardt 2006). If the recommendations are not applicable, in an investigator's considered judgment, then he or she should focus on how they can be modified to fit their situations without compromising ethical concerns. Collecting or study permits issued by relevant local, state, and federal agencies or appropriate offices in foreign countries should be submitted with protocols to IACUCs whenever possible. Approval by these agencies may assuage concerns about the impact of field research on natural populations.

IACUCs often identify problems and then propose solutions for the investigator to consider. This is often useful when the researchers and veterinarians on the committee are familiar with the subject species and nature of the research questions. At most institutions such knowledge is far more available for traditional mammalian laboratory species and biomedical research than for reptiles and field research. Thus, committees may identify problems but not have solutions at hand. For this reason, investigators should consider providing more background and supporting information that anticipates concerns brought by those from a background focused on laboratory studies of endotherms.

What Do We Know About How Reptiles Experience Their World?

Even today, many of those devoting their professional careers to studying ectothermic reptiles, in contrast to those studying birds and mammals, have a limited appreciation of the flexibility of their behavior and psychology. This may result in the use of methods without careful evaluation; such attitudes may carry over to research teams and field assistants. This seems to be, in part, a consequence of the fact that reptiles lack many of the traits (e.g., facial expressions, squeals) that we, as mammals, associate with pain, suffering, and stress (Bowers and Burghardt 1992). To some extent, the attitudes of herpetologists mirror those of the public. Survey studies of college students found that they view reptiles as fairly low on measures of cognitive ability, sentience, and affect compared to mammals and birds, although turtles ranked above snakes (the only two reptiles included) on all three measures, and even above rodents and bats on some (Herzog and Galvin 1997). Attitudes about "nice" and "bad" animals certainly operate here with the public; one would hope that professionals would not harbor similar biases.

A consequence of the above is the enduring legacy of methods developed in an era when few scientists thought that reptiles had complex sentient or social lives. This view has certainly been modified today in our recognition of complex social behavior, communication systems, parental care, and other advanced phenomena in crocodilians and many lizards; however, social systems in turtles and snakes are still often considered rudimentary. The abilities of reptiles to learn, experience emotions, and be affected by ontogenetic experiences have also been underestimated. Recent experimental demonstrations of rapid problem solving in lizards and spatial learning in turtles illustrate the capacities they may have. Both field and laboratory studies show that many turtles have very long memories, commensurate with their longevity. To take seriously the emotional, social, and cognitive lives of reptiles is neither anthropomorphic wooly-mindedness not fit for scientific concern, as is often thought, nor something to be repressed in order to avoid seriously considering the consequences of recognizing animal experiences (Rollin 1989). However, a critical anthropomorphism is possible in which natural history, physiology, experimental data, and our own experiences as sentient beings are used to identify possible troubling procedures that would not be deemed appropriate with other amniote vertebrates (e.g., Morton et al. 1990). For example, the time scale of behavior and lower metabolic rates in ectothermic reptiles may hide phenomena from us that we uncritically accept when seen in a species biologically more similar to our own (Rivas and Burghardt 2002). In fact, the entire issue of animal sentience and consciousness in regards to ethics is continually shifting (Burghardt 2009).

Reptile behavior may often seem fairly stereotyped, but the capacity of these animals for learning has been underestimated. Even evidence available more than 25 years ago showed that the cognitive abilities of reptiles converged on and overlapped with those of many mammals (Burghardt 1977). Currently, much more evidence is available for the ability of reptiles to learn many things in the laboratory and in the field. In fact, failures of reintroductions in new habitats can be attributed to the animals' unfamiliarity with the area and habitat. Reptiles may develop food preferences and need experience in hunting, capturing, handling, and swallowing different food types. Reptiles may need to develop cognitive maps of their home areas in order to escape predators, defend territories, find mates and nest sites, and so forth, adequately.

What about the abilities of reptiles to show signs of affect when stressed or injured? In many respects this is not something that we can directly ascertain. But methods can assess emotion (e.g., Cabanac and Cabanac 2000), play (e.g., Burghardt et al. 2002), stress (e.g., Romero and Wikelski 2002), and other attributes not typically associated with reptiles, and more are being developed. In the future, brain-imaging studies will allow more precise interpretations. For now, being aware of recent advances in reptile mentality and physiology, and adjusting field methods accordingly, is appropriate. To establish that a manipulation does not have detrimental effects is as important as finding the reverse. Indeed, it may be better to prevent harm in advance.

Some Specific Ethical Issues in the Field

In the context of the discussion above, it is important for investigators to consider the following 10 questions about their fieldwork methodologies, even if the issues are not formally considered in research protocols or designs.

1. Do the methods for locating and collecting animals destroy the habitat for the target and/or other species?
2. Does capturing and measuring the animal cause it undue stress or affect its subsequent behavior if it is released?
3. Do marking and/or tracking methods alter behavior, survivorship, or social interactions?

4. Are surgical procedures such as the implantation of a radio transmitter based on current best practices for anesthesia, surgery, recovery, and release?
5. Will the number or type of animals removed from a site affect the reproductive potential, social structure, or population viability of the species, and are such effects acceptable?
6. Are methods of field preservation designed to minimize stress and suffering of the subject animal(s) from capture to death?
7. Are all precautions taken to avoid introduction of pathogens or parasites into the native habitat and population?
8. Are methods of transport such that temperature, food, water, and other needs can be met beyond the bare minimum for survival?
9. Are temporary holding facilities for processing animals arranged to eliminate disease transmission, parasites, stress, and injury?
10. Do reintroduction studies monitor survivorship if not the actual movements and behavior of a significant sample of the animals released into new habitats?

Some Ethical Issues in the Laboratory

Although this volume is mostly devoted to fieldwork, such work may have captive components. Ethical aspects of research on captive animals begin with their housing and maintenance. Great progress has been made in reptile husbandry and veterinary medicine in recent years. Individual and species differences in behavioral needs and temperament need to be evaluated before keeping diverse species with which a researcher may not be familiar. Compendia such as Warwick et al. (1995) and herpetoculture magazines often provide useful information for maintaining animals for captive study and should be consulted, especially when large numbers of animals are to be cared for. In this rapidly changing field, few answers will remain certain. As mentioned above, a growing literature is indicating that reptiles, like mammals, may need many types of environmental stimulation, not only to maintain good captive health and normal behavior, but also to promote physical growth, behavioral development, and acquisition of species-typical behavior. The last factor is particularly important if captive bred or produced offspring are used in reintroduction programs.

Acknowledgments

I thank those who have aided my thinking on animal issues and ethics over the years, including Harold Herzog, Marc Bekoff, Strachan Donnelley, and Lilly-Marlene Russow.

Handling Live Reptiles: Leave Your Ego at the Door

Steven J. Beaupre and Harry W. Greene

A Comment on Responsibility

Venomous snakebite is a serious health threat, and bites from large species can be permanently disabling. Under the best of conditions, a person bitten by a venomous snake can expect extreme pain and several weeks of recovery. Under the worst conditions, a bite from a large rattlesnake can be life-threatening or permanently disfiguring. All would agree that the consequences of a lapse in safe handling technique can be horrifying.

As scientists, we depend upon the good will of funding agencies to support our research programs. We can expect that such agencies (and peer reviewers) will scrutinize research proposals involving hazardous species intently, especially those that involve students. Even among professional biologists, the basic natural history and behavior of venomous snakes are largely misunderstood (Beaupre and Duvall 1998). With each illegitimate bite (i.e., one involving purposeful exposure to risk) or injury incurred by researchers studying dangerous organisms, the credibility of the group erodes among its peers. Researchers that utilize potentially dangerous species have a responsibility to their students and colleagues, to themselves, and to the broader herpetological community to conduct their work as safely and humanely as possible.

General Safety Principles

Most reptiles are harmless and can be handled safely with attention only to animal well-being and prevention of disease through routine hygiene (Greene 1995). A great variety of methods for handling specific species have been published in *Herpetological Review,* and we recommend that investigators search that journal, in particular, prior to initiating any new study. Some reptiles are dangerous (including large and venomous species) and can injure humans and themselves unless handled with extreme care. Most of the information contained in this this section is common sense to many herpetologists, but it may be invaluable to those considering working with hazardous species for the first time. Although a wide array of techniques and equipment for handling hazardous species are available, a cautious attitude and awareness may be more valuable. We stress here that exercising maximum care and remaining alert are critical to a safe working environment. Successful and safe handling of dangerous reptiles can be achieved only by the simultaneous application of the following three elements.

1. Attention: Reptiles are wild animals, and as such, they are unpredictable. Investigators must always concentrate and focus their attention on the animal being handled. They should never work with dangerous live reptiles under distracting circumstances and should always assume that an animal will react defensively, potentially injuring someone.
2. Equipment: Using equipment such as tongs, tubes, squeeze boxes, and noose poles places a barrier between an investigator and the animal. A barrier is critical to safety, but it is rarely sufficient by itself. For example, some herpetologists handle small venomous snakes when wearing heavy leather welding gloves. The practice has resulted in several illegitimate envenomations when fangs unexpectedly penetrated the leather. The gloves provide a false sense of security, and when they fail, the result can be a bite that threatens the life of the investigator either through the direct effects of the venom or because of an acute allergic reaction.

3. Distance: Attention and equipment alone are not foolproof if they lapse. Distance, however, is sure to prevent accidents. A handler should always keep a dangerous reptile at a safe distance from his or her body and extremities, even when the animal is controlled by equipment.

In addition, one should never work with dangerous reptiles when he or she is physically or mentally unwell (sick, depressed, or emotionally distracted) or under the influence of drugs or alcohol.

Handling Venomous Snakes

COLLECTION

Safety with venomous snakes begins in the field during collection. When working in areas where venomous snakes occur, one should never put any part of his or her body in places that have not first been visually inspected. Placing one's hands in burrows or under rocks is obviously hazardous, but sitting on a log could also be dangerous if a timber rattlesnake in hunting posture is on the other side! Wearing high, sturdy, leather boots is always advisable, and snake-proof gaiters or chaps (new lightweight equipment made of Kevlar is now available commercially) provide an additional level of protection that may be especially desirable in some habitats. Manually restraining venomous snakes (i.e., "pinning and necking" the snake with the bare hand) is rarely, if ever, justified, and doing so is gratuitously dangerous for both the snake and the handler. Instead, a hook or tongs should be used to pick up and control the animal. Most (but not all) commercially available snake tongs are designed to provide a grip that is strong enough to hold the snake but not strong enough to hurt it (Fig. 37). Snakes should be grasped between the front third and middle of the body (i.e., just forward of the center of the body). Vipers, in particular, are heavy bodied, and if grasped too far forward, may experience serious spinal injury from stress produced by their own weight. Snakes should be lifted and moved slowly to avoid undue stress on the spine. If a snake begins to thrash while being moved with tongs, the investigator should maintain her grip with the tongs but place the snake back on the ground. After the snake settles down, another attempt can be made to move it. A snake being moved with tongs or a hook should always be held away from the collector's body and the bodies of other people. When working with snakes, it is important to be on stable ground with good footing, even if that requires carrying a snake with tongs to a level and stable substrate.

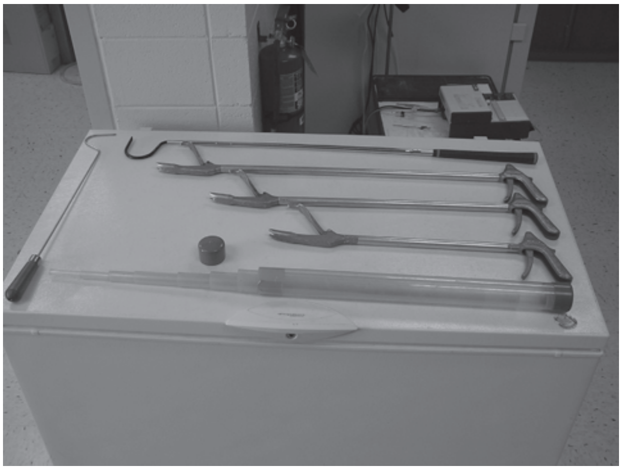

FIGURE 37 Commercially available tools for handling venomous snakes. Top and left: hooks of different sizes; Center: snake tongs in a variety of sizes; Bottom: set of eight transparent snake tubes. (Photo by S. J. Beaupre.)

TRANSPORT

Traditionally, snakes are transported in snake bags or in modified 5- or 10-gal buckets. Snake bags are usually nothing more than long pillow cases, but superior bags can be purchased or easily manufactured. They should be of light but sturdy cotton, with double stitching in bright thread (so that tears are visible). The corners of the bag can be sewn across, preventing access by the snake and providing safe handles for grasping to up-end the bag when the snake is removed. A snake can be maneuvered into a bag by a variety of techniques; perhaps the safest is to hold the bag open at a safe distance with a hoop on a pole while the snake is placed in the bag with tongs or a hook. The snake is forced down into the bottom of the bag and trapped by laying the snake hook or tongs across the bag. The open end of the bag is twisted and tied in a knot. The main advantage of a snake bag is portability; it is light, and it folds into a small space. With practice, a collector can use one easily and safely as long he carries an occupied bag away from his body (snake fangs can easily penetrate cloth bags). Perhaps one drawback of many snake bags is that the contained snake is not visible; collectors must exercise extra caution when the snake is manipulated in or removed from the bag.

Buckets are a relatively safer alternative to snake bags. Usually, a 5- or 10-gal plastic bucket is modified by placing escape-proof screen or hardware cloth windows in the lid and/or bottom for ventilation (alternatively, simple ventilation holes can be made with a drill). The lid can also be fitted with a handle near the center, which allows the collector to place a lid on the bucket without exposing his or her hand to the snake. Buckets are bulkier than snake bags, but a single bucket can sometimes safely carry more than one snake (depending on body size), no extra equipment is required to hold the bucket open, and the snake remains visible even when enclosed. Buckets are also rigid in construction, which reduces the chance of crushing during transportation. One should never put his or her face close to a bucket containing venomous snakes; the screen lids of the buckets are present for ventilation only, not as windows. Snakes under stress may strike at the window. A properly secured bucket will prevent a bite, but the snake may spray a mist of venom into the face and eyes (Gans and Taub 1964). Likewise, neither the hands nor any other part of the body should be placed on the screen lid of a bucket or cage that contains a live snake. In general, when transporting snakes care should be taken to ensure that bags or buckets are kept cool, and even damp. Snakes are very sensitive to heat and will die quickly if left even for a few minutes in direct sunlight or in a hot, closed vehicle.

IMMOBILIZATION

Frequently, research projects require that snakes be immobilized for sexing, taking blood or tissue samples, administering

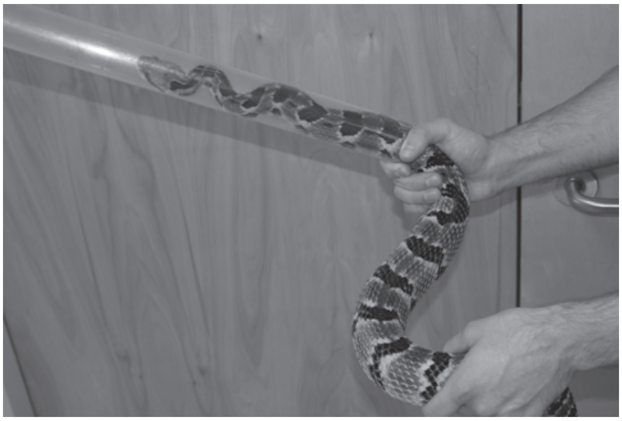

FIGURE 38 Timber Rattlesnake (*Crotalus horridus*) safely immobilized in a transparent plastic tube. Note that the tube is small enough to prevent the snake from reversing direction. The operator grasps the end of the tube with the thumb and index finger, and the body of the snake with the remaining three digits, thus locking the snake and tube in a fixed position. The tube is comparatively long so the snake cannot emerge at the opposite end. Once secured, researchers can safely access the posterior of the snake to assess reproductive condition, determine sex by caudal probe, or draw blood. (Photo by S. J. Beaupre.)

anesthesia, or euthanasia. Perhaps the most widely used and safest method (when properly applied) for immobilizing venomous snakes is the use of clear plastic, Plexiglas, or Lexan tubes (Fig. 38; Murphy and Armstrong 1978). Tube diameter should be sufficient to allow the snake to enter but not to turn its head and reverse direction. A tube should also be long enough to ensure that as the end of the tube is presented to the head of the snake, the operator's hand and other body parts are well out of striking range. Coaxing a snake into a tube is usually accomplished by first placing the snake on open floor (with tongs or a hook), placing the end of the tube over the head of the snake, and gently tapping the snake at various posterior points until it willingly crawls into the tube. In some cases, a snake's motivation can be enhanced by placing it in a bucket or garbage pail containing 10 to 20 cm of water. The snake will attempt to escape up the side of the bucket and can be more easily coaxed into the tube. After a snake has advanced into the tube sufficiently (with at least the anterior third or half of the body safely inside the tube), the investigator grips the tube and snake simultaneously with a single hand, thus locking the snake in place. The posterior portion of the snake can now be accessed safely for injection, tissue sampling, or other procedures. If a snake is large, handling may require two people, one to secure the snake in the tube and the other to carry out the desired procedure. It is important to ensure that the snake cannot crawl through the tube and emerge head first at the opposite end (one end can be plugged to prevent this) and that the snake cannot fall out of the tube leaving the operator holding a live snake by the tail or mid-body. The latter consideration is especially important for very small snakes. A person holding a tubed snake must never lose visual contact with the snake; even a few seconds of inattention can result in a bite.

MEASUREMENT

Many research programs require that the routine measurements of the snake, such as standard lengths and body weight, be taken. Snakes and beaded lizards can be measured effectively in a "squeeze box" (Quinn and Jones 1974). A squeeze box immobilizes an animal by pressing it between a transparent lid and a soft foam bottom. Squeeze boxes have diverse designs (e.g., Cross 2000), but basically, they are all modifications of the original described by Quinn and Jones (1974). A squeeze box is usually four-sided, with a bottom, and made of wood (Fig. 39). The bottom of the box is lined with a soft foam pad. The squeeze box lid is constructed of Plexiglas with wooden handles and fits within the box walls. Usually, one side of the box is modified with a port to allow access, either by cutting a large hole at the level of the foam pad or by cutting a trough down the center of the wall to allow the tail of the snake to hang out. The animal is placed on the foam bottom; the Plexiglas is lowered until it is in contact with the dorsal surface of the animal. The animal is then immobilized by being pressed into the foam pad.

Morphological landmarks can then be traced on the transparent surface with a water-soluble (nonpermanent) marker and subsequently measured directly from the Plexiglas using a flexible measuring tape. In addition, the tail of the animal can extend out of the access port so that PIT tags can be injected, blood or tissue sampled, measurements taken, and other procedures carried out safely. A portable squeeze box for field use can be constructed from lightweight luggage or carrying cases. The first author (SJB) uses a field-portable squeeze box constructed from a discarded video recorder carrying case. He stripped the inside of the case bare, placed a foam pad in the bottom, and made a Plexiglas lid with small handles. The box is light, easy to use, and provides carrying space for a variety of tools (e.g., spring scales, syringes, blood collection tubes, snake tubes, and bags).

Live weights of venomous reptiles can be obtained in a variety of ways. In the field, it may be most effective to confine a snake in a bag of known weight, obtain the weight of the snake and bag using a spring scale, and then calculate the weight of the snake by subtraction. The use of spring scales in the field trades convenience for precision. In the laboratory, snakes are weighed most effectively with a digital balance and a large weigh container with a lid. The weigh container is placed on the balance, and the balance is zeroed. The snake is then coaxed into the weigh container, which is then covered with the lid delivered from a distance with snake tongs. The weigh container with the snake is then transferred to the balance pan. With some practice, this technique can be very safe even with fairly large snakes. Some researchers use vapor anesthetics to induce a deep plane of anesthesia in snakes rather than working with conscious, alert individuals (Gans and Taub 1964; Fitch 1987; Hardy and Greene 1999, 2000; also, see next section, "Anesthesia in Reptiles"). Currently, a popular and relatively safe agent is the volatile gas Fluothane (Gans and Taub 1964). Unconscious snakes can be weighed and measured with minimal risk to the investigator. Snakes may be at some risk from overdoses or adverse reactions to anesthetics; with practice, however, those risks can be minimized. The primary drawback of using anesthetics is the additional time required, although that cost is easily balanced by increased safety for researchers and snakes. For studies in which few snakes are processed, added time poses little problem. If large numbers of snakes must be processed, anesthesia may prove impractical.

Herpetologists employ various techniques when measuring the lengths of snakes, including using a squeeze box, stretching the animal against a ruler or meterstick, and administering

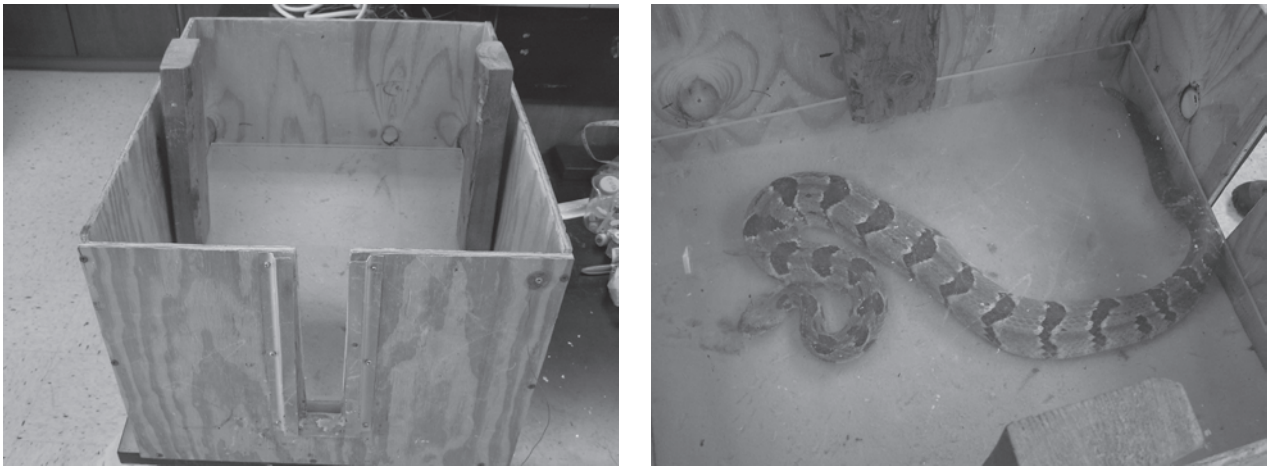

FIGURE 39 Basic squeeze-box design and operation. Left panel: Showing 4 wooden walls, soft foam in floor of box, transparent ¼-inch Plexiglas lid with two 2- by 4-in handles, and slot for accessing the snake's tail while the anterior half is pinned in the box. A small door (not pictured) slides down the grooves at the slot to close the box completely when desired. Right panel: A Timber Rattlesnake (*Crotalus horridus*) is pinned between the transparent lid and foam pad for landmark placement. From this position, the tail can be draped through the slot for safe access. (Photo by S. J. Beaupre.)

anesthetics to render the animal unconscious. Each technique carries a unique measurement error, the relative magnitudes of which have not been quantitatively assessed. Squeeze-box measurement error is usually less than ±1 percent of total length as determined by repeated measurements of the same individual (Quinn and Jones 1974; Brown 1991; Beaupre 1995). Measurement errors associated with stretching the animal against a meterstick (for safety reasons, we do not recommend this practice) may be large (5–10% of total length), because of lack of cooperation by the snake and the flexibility of connective tissue and musculature that support vertebral column movement (D. Cundall, pers. comm.). The spine musculature and connective tissue may also relax under anesthesia such that total length of an animal may differ when conscious and unconscious. We recommend that investigators select a safe technique (squeeze box or anesthesia) and employ only a single technique in each study to enhance internal consistency.

Housing and Maintenance

Because housing and care of venomous snakes have been discussed in detail elsewhere (Gans and Taub 1964; Murphy and Armstrong 1978; Murphy and Campbell 1987), we consider only some special areas of concern from the standpoint of safety. Needless to say, venomous snakes should be housed in escape-proof cages, and meticulous care should be exercised to ensure that cages are secured properly after feeding, cleaning, and other procedures. Colonies should be housed in locked, escape-proof rooms, and access should be restricted to trained personnel (Gans and Taub 1964); we specifically exclude custodial and physical plant staff. Safe procedures for routine operations such as cage cleaning and feeding should be established. An absolute ground rule is that hands are never placed within striking distance of the opening of a cage or into a cage containing a venomous snake. Venomous snakes are always moved to "shift containers" (other cages, buckets, or locking garbage cans) during cage cleaning. In addition to the general safety precautions already outlined, we recommend that workers protect their eyes with safety glasses when handling or transporting snakes in small spaces or when in proximity to, or carrying, cages with screen lids to minimize the risk of incidental venom spray (Gans and Taub 1964). Furthermore, all reasonable effort should be made to minimize direct contact between venom and skin. It is possible (although unstudied) that periodic cutaneous exposure to venom may increase a researcher's risk of severe allergic reactions in the event of a bite. Finally, a written protocol for evacuation of a snake bite victim to a medical facility should be established, and all personnel should be trained in appropriate response in the event of such an emergency (e.g., Gans and Taub 1964).

Techniques to Avoid When Handling Venomous Snakes

NOOSES AND NOOSE TUBES

Nooses in general are designed to snare a snake around the body or neck and hold the snake for control. By their nature, nooses are dangerous both to the animals and to the researchers. Nooses apply force to the spine or neck of the snake over a very small area. If a noosed snake is lifted from the ground or thrashes, the force applied is easily enough to dislocate a vertebra or sever the spine. In addition, releasing the snake from the noose may be problematic depending on the design of the noose. A noose tube consists of a small movable metal pipe installed inside a larger Plexiglas tube. A line with a noose at the end runs through the pipe; it can be drawn tight around the neck of a snake so that the snake can then be dragged into the Plexiglas tube (see King and Duvall 1984). The noose tube also relies on the application of high force over a small area and may injure the snake.

NECKING AND/OR TAILING

Herpetological tradition, as well as the activities of recent television personalities, has presented the handling of venomous snakes with bare hands as an acceptable technique. To neck a snake the handler pins the animal's head to the ground, reaches down and grabs the animal behind the head, and holds it at the neck–head interface with a firm grip (see Fitch 1987). Although many herpetologists have successfully used this technique for years, we do not recommend it, because it violates the distance criterion (see "General Safety Principles," above). Numerous well-known cases of illegitimate bites can be attributed to human error during the pinning and necking process. Some individuals grasp venomous snakes by the tail, relying on gravity to keep the snake from turning to bite them. Tailing snakes in this fashion is an extremely dangerous practice, because the head is neither controlled nor isolated from the handler by equipment or distance, thus violating both equipment and distance recommendations (see "General Safety Principles," above). Several species of venomous snakes (e.g., most rattlesnakes) are strong enough to lift themselves easily into striking range of the hand, arm, or body of the handler. Furthermore, the cardiovascular systems of aquatic species and many terrestrial species (especially large vipers) are poorly adapted to cope with vertical postures and gravitational forces, which can cause negative pulmonary effects and blood pooling (Lillywhite and Smits 1992). We agree with Gans and Taub (1964) that there is little if any justification for handling venomous snakes using either of these techniques.

GLOVES

The use of heavy leather welding gloves for handling small venomous snakes is another common technique. Such gloves can even be purchased through herpetological supply houses. Although it seems reasonable that bites on the hands from small species can be prevented by using adequate protective gauntlets, in practice several envenomations have occurred. Occasionally, a snake gets lucky and penetrates a glove at the seam or in a weak spot. The primary problem with gloves is that the researcher relies 100 percent on their efficacy. The handler does not keep the animal at safe distance or control the head, and because the gloves offer a false sense of security, his or her attention can stray. The false sense of security may also cause the handler to rely on gloves when handling increasingly larger snakes. No data-supported recommendations regarding the species and size range of snakes that can be "safely" handled using gloves are currently available. We view this technique as dangerous and unpredictable.

Handling Large, Biting Species

Many reptiles, although nonvenomous, are large enough to deliver an injurious bite or scratch. Such animals require special restraint, the details of which vary according to species. Most large turtles, for example, can deliver a serious or even disfiguring bite, and their sharp claws can scratch. Large turtles should be handled by the shell to avoid the jaws and feet (see Conant and Collins 1998, fig. 1; and Fowler 1978, figs. 25-14 through 25-18). Methods for handling several large turtle species are reviewed in Fowler (1978). It is possible to safely immobilize a large anaconda (*Eunectes* sp.) by covering its head with a sock and securing it with electrical tape (Rivas et al. 1995). Crocodilians, which are especially large and powerful, can be captured and handled using a variety of techniques (see Chapter 5, especially "Finding, Counting, and Catching Crocodiles," and Chapter 11, "Swamp-Dwelling Reptiles"), including nooses, snares, ropes, nets, and squeeze cages (Fowler 1978). Modern capture and transport devices (crocodilian tubes) have been constructed from PVC pipe (Jones and Hayes-Odum 1994) or plastic drainage pipe (Saumure et al. 2002). The use of tubes for handling crocodilians does not differ fundamentally from the use of tubes in handling snakes, except that the tubes are larger and longer, and usually enclose the entire animal. A crocodilian can be noosed at the head and pulled into the tube (Jones and Hayes-Odum 1994), or it can be coaxed or prodded gently from behind (Saumure et al. 2002).

Handling Delicate Species

Handling small and delicate reptiles securely and without injury can also be difficult. The method most appropriate to use will vary according to the species. As a generalization, small lizards and snakes that are held too loosely or between cupped hands can escape by squirming, whereas those that are restrained on the body between thumb and fingers may be injured if they struggle. Many small species of limbed lizards can be gently but firmly grasped by the femoral region of a hind leg; if allowed to turn and grasp a nearby finger, a lizard will sometimes sit quietly while held. Care should be taken not to pin or pinch regions of the animal that harbor delicate vital organs. Collectors should never grasp the tail of any small lizard, because of the high probability of *tail autotomy* (self-determined loss of a portion of the tail) in some lizards (e.g., geckos, skinks), which allows the animal to escape from predators; the tail continues to move after detachment, attracting the attention of the predator as the lizard escapes). Small species also have a high surface-area to volume ratio, which makes them especially susceptible to overheating and desiccation. Even body heat from an investigator's hand can rapidly induce heat stress in some species. In dry or hot environments, collectors should dampen capture bags to reduce gradients that drive evaporative water loss and ensure that the animals have an adequate water supply while in captivity.

Closing Remarks

In this section, we have described only the fundamentals of handling potentially dangerous and delicate species. We have attempted to provide access points to the primary literature, and we encourage the reader to research techniques in detail, prior to initiating a study. Various other sources, not cited above but relevant to issues of animal care and safety, include Altimari (1998), Pough (1991), and the ASIH Guidelines (2004). They also provide more detailed access to the literature. We stress again that the safety of investigators and animals is a professional responsibility of all who engage in reptile research.

Anesthesia in Reptiles

Dale DeNardo

A vast variety of anesthetic agents have been used in reptiles. In part, this reflects the lack of a single, good universal anesthetic regimen that is safe and effective for the existing diversity of species and procedures. Frequent problems associated with reptile anesthesia include long induction and recovery times, a high degree of intraspecific and interspecific variability in the anesthetic effect, and difficult means of anesthetic delivery. These problems are exacerbated in the field, where returning animals back into their environments as rapidly as possible is critical to their survival and where heavy equipment for administering anesthetics and/or regulations associated with federally controlled substances are difficult to accommodate.

The "Perfect" Anesthetic

While nothing is ever perfect, it is beneficial to explore what the characteristics of a perfect anesthetic for reptiles might be. One can then critically assess the various anesthetics available and choose the one that best fits the species and specific goals of the study.

EASE OF DELIVERY

The use of a simple anesthetic regimen will reduce the time necessary to train individuals in its use and, more importantly, reduce the likelihood and frequency of user error (i.e., improper implementation of the anesthetic protocol rather than an inherent problem with the protocol itself). Many reptiles do not have readily accessible veins, so administration of intravenous anesthetics, such as propofol (Diprivan), requires a great degree of expertise; in addition, the possibility of extravascular administration increases the risk of poor anesthesia. Similarly, the novice may find standard equipment used with gas anesthesia difficult to master.

EXPENSE

While low cost should not be a high priority when selecting an anesthetic, it understandably plays a role. High costs may prohibit the use of gas anesthetic machines or limit the choice of drugs. When cost is used in the decision process, investigators must be certain that the less costly option does not substantially increase the risk of anesthetic complications. Costs associated with the inadvertent death of an animal from anesthetic usually far outweigh any savings associated with the use of a more economical anesthetic regime.

EFFICACY

Effects of anesthetics vary. Some agents produce deep anesthesia, whereas other agents induce only a sedated state. Although the immobilization and analgesic effects of an anesthetic agent are often assumed to be linked, that is not always the case (e.g., an animal can become immobilized but still perceive pain). A good anesthetic agent should provide both immobilization and analgesia. Additionally, the duration of the anesthesia induced is quite variable among agents, doses, and species. Therefore, it is crucial to choose an anesthetic regimen that provides the appropriate level of immobilization and analgesia for the duration of the procedure.

SAFETY

The most important trait influencing the choice of anesthetic agents should be the safety of that agent for the target animal. The survival rate after anesthesia should be virtually 100 percent; the high quality of anesthetic agents and supportive care currently available mandate nothing less. Mortality due to anesthesia should be considered unacceptable, and the anesthetic protocol or individual training should be modified if deaths occur. Currently, the safety of anesthetic agents used in reptiles varies widely. New anesthetic protocols proposed for use on critical animals or in critical situations should be tested before implementation.

CONSISTENCY

The consistency of a drug's anesthetic effect is important and often reflects the safety of the agent. Optimally, an anesthetic agent should provide similar effects among different animals of the same species. However, some anesthetic agents vary greatly both within and among species. A given dose may induce effects ranging from light sedation to surgical anesthesia to anesthetic overdose. Such agents must be used with extreme caution, often with stepwise increases in the doses delivered.

INDUCTION RATE

Optimally, an anesthetic protocol should quickly induce the desired plane of anesthesia in the animal. In reptiles, induction times with some agents can be extremely long, which limits productivity and may lead to improper levels of anesthesia. Slow-acting anesthetics can tempt a surgeon to begin a procedure before the animal reaches an appropriate level of anesthesia, or to supplement the initial dose with additional anesthetic, which can ultimately lead to an overdose once all the administered drug reaches peak effect. This latter concern is especially problematic when using slow-acting injectable anesthetics.

PHYSIOLOGICAL EFFECTS

The ideal anesthetic regime induces the desired plane of anesthesia with little to no effect on other physiologic function. However, all anesthetics generally have undesirable side effects. Most of them suppress the cardiovascular or respiratory systems, or both, to some degree (Custer and Bush 1980; Schumacher et al. 1997; Bennett et al. 1998; Sleeman and Gaynor 2000). Substantial suppression of cardiopulmonary function can be clinically significant and jeopardize the well-being of the patient. Even slight suppressive effects that are clinically irrelevant can be important if investigators are collecting physiological data from anesthetized animals. While the cardiovascular and respiratory systems are of primary interest, anesthesia can also affect other systems in some species, such as the auditory system of *Gekko gecko* (Dodd and Capranica 1992). Therefore, when selecting an anesthetic agent, one must consider not only the effectiveness and safety of the agent but also its potential effect on any physiologic data being collected.

RECOVERY RATE

The rate of recovery from anesthesia is usually considered more important than the rate of induction. Recovery rates for different anesthetic agents range from minutes to days. If rapid recovery shortens the duration of the anesthesia, then it reduces risk to the animal and allows the investigator to move on to the next animal, improving his or her productivity. The point at which an animal is considered to be recovered varies. Minimally, an animal should be ambulatory and breathing normally. However, such a state of recovery is insufficient for animals to be released into the wild. Those animals not only must be able to ambulate, but they also must be able to move swiftly and with good coordination in order to avoid predators and, in some cases, to defend themselves from conspecifics.

Anesthetic Agents

GAS ANESTHETICS

Gaseous agents are the anesthetics most widely used for reptiles. Anesthetic delivery and, therefore, the level of patient anesthesia can be easily adjusted (see Bennett 1996). In addition, the anesthetic effects of these agents are relatively consistent, so gas anesthetics have a high margin of safety. While agents vary, gas anesthetics have relatively short induction and recovery periods compared to most injectable agents.

The biggest drawback of gas anesthetics is the complexity of the optimal delivery system. These agents should be delivered via an anesthetic machine that uses a precision vaporizer to deliver a carefully regulated concentration of anesthetic in oxygen. Upon exposure to a gas anesthetic, reptiles often stop breathing for extended periods, which greatly increases induction time. In addition, because the glottises of awake reptiles remain closed between breaths, gaseous anesthetics can only be delivered during breaths. Placing an endotracheal tube in the patient assures a steady delivery of the agent to the lungs. Once the animal is anesthetized, the glottis relaxes in an open position.

Access to the glottis for intubation varies among taxa. Snakes, in which the glottis is located toward the front of the mouth, are easily intubated. Intubating a turtle can be much more difficult because it can retract its head into its shell, it typically has strong jaws, and the glottis is located behind the fleshy tongue. In addition, the small diameter of the trachea in small reptiles, including snakes, may preclude intubation.

Endotracheal tubes must always be of appropriate diameter and length. Excessively long tubes may have a dead space volume that exceeds tidal volume, causing carbon dioxide and exhaled anesthetic gas to be re-inhaled. The presence of carbon dioxide in inspired air reduces overall ventilation in anesthetized reptiles, compromising respiratory capacity (Furilla and Bartlett 1989).

In addition to increasing the speed of induction, intubation hastens recovery. Commonly used gas anesthetics are cleared predominantly through the lungs. Therefore, respiratory suppression associated with anesthesia can slow the elimination of anesthetic from the body. Ventilating the patient—the investigator inserts a small endotracheal tube through the glottis and blows air into the lungs—hastens recovery. During such a procedure, the investigator must visually monitor inflation of the body cavity to assure that the lungs are not overinflated.

In many cases (e.g., small animals, field conditions, limited budgets), use of an anesthetic machine is not feasible. However, gaseous anesthetics can still be administered. An inexpensive gas delivery system can be made from readily available materials–two standard laboratory squeeze bottles, ¼-in diameter vinyl tubing, a latex glove, and a rubber band (Fig. 40). By squeezing the anesthetic-containing bottle, anesthetic is delivered to the animal without investigator exposure to the drug. Such systems do not regulate the vapor pressure of the anesthetic, so the potential for anesthetic overdose is greater. However, such risk is minimized by using a head mask rather than intubating the patient. Deeply anesthetized reptiles have a greatly reduced respiratory rate, so that increased anesthetic depth leads to reduced alveolar exposure to the anesthetic agent in nonintubated patients. Intubation bypasses this inherent control. While suppressed respiratory rate also reduces oxygen delivery to the alveoli, potential clinical complications associated with hypoxia are unlikely because of the extremely low metabolic rate of anesthetized reptiles.

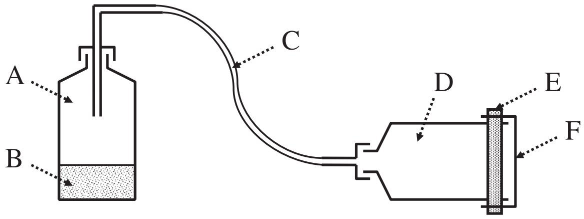

FIGURE 40 Diagram of a simple yet safe and effective closed-system anesthesia device. Materials include: (A) laboratory squirt bottle with inner plastic straw cut in half; (B) isoflurane; (C) vinyl tubing; (D) squirt bottle with bottom cut off, nozzle cut short, and inner plastic straw removed; (E) rubber band; (F) piece of latex with a hole in the middle (cut to a size that assures a snug fit around the patient's neck). Note: This unit does not control vapor density, leading to very high percent isoflurane levels; it should never be used on an animal that has been intubated with an endotracheal tube (see text for further discussion). (System adapted with permission from one used in the laboratory of L. J. Guillette, Jr., University of Florida, Gainesville, Florida.)

As a result of masking rather than intubating the patient, induction takes longer (due to breath holding), and the level of anesthesia is not as consistent. Nevertheless, the procedure is extremely safe and effective for a wide variety of small- to medium-size reptiles. This system is inadequate for larger reptiles, whose ventilatory volumes and levels of oxygen consumption are both greater. The primary advantage of this delivery method over a simple induction chamber (i.e., anesthetic applied to cotton and placed in a container with the animal) is that it greatly reduces exposure of the researcher to the anesthetic gas and permits anesthesia to be maintained during the procedure.

Traditionally methoxyflurane (Metofane) and halothane (Fluothane) were the primary gas anesthetics. They have largely been replaced by isoflurane (Forane), which has a similar level of patient safety but has substantially reduced induction and recovery times. Sevoflurane, a relatively new gas anesthetic, is gaining popularity in veterinary medicine. For reptiles, it provides safe and effective anesthesia with rapid induction and recovery times (Rooney et al. 1999; Bertelsen et al. 2005). It also lacks the unpleasant smell of isoflurane that can cause patient breath holding and therefore longer induction times for animals that are not intubated. However, in Green Iguanas (*Iguana iguana*), there is no detectable difference in the pharmacokinetics of isoflurane, sevoflurane, and desflurane, another newer gaseous anesthetic (Brosnan et al. 2006).

In addition to the anesthetic, the source gas can also vary. While 100 percent oxygen is typically preferred for mammalian anesthesia, the use of room air (approximately 21% oxygen) may be equally effective in reptiles. Interestingly, anesthesia with sevoflurane in 100 percent oxygen suppresses ventilatory rate more so than anesthesia using sevoflurane in room air. However, oxygen concentration does not affect recovery time (Bertelsen et al. 2005).

INJECTABLE ANESTHETICS

Investigators and veterinarians have administered a variety of injectable anesthetics to reptiles, but ketamine hydrochloride is the most widely used. Earlier literature promoted it as an effective anesthetic for reptiles (Glenn et al. 1972; Cooper 1974; Harding 1977; Font and Schwartz 1989), although numerous significant complications associated with its use have been reported, and some individuals recommend against its use (Throckmorton 1981). The anesthetic effects of ketamine can be quite variable both within and among species. Similar doses can lead to effects ranging from ineffective sedation to surgical anesthesia and death (Cooper 1974; Harding 1977). Ketamine doses generally range from 20 to 60 mg/kg body weight, although doses in excess of 200 mg/kg have been required for adequate anesthesia of some reptiles (Cooper 1974; Arena et al. 1988). Those higher doses are well above the LD_{50} dose of 154 mg/kg body weight determined for *Crotalus atrox* (Glenn et al. 1972). In addition, ketamine can have an extremely long recovery time (up to 7 days; Glenn et al. 1972) making it impractical for many studies. Lastly, the U.S. Drug Enforcement Administration (DEA) lists ketamine as a schedule IV controlled substance, and as such, the acquisition, storage, and use of ketamine is tightly regulated. A purchaser must have a DEA license, the drug must be stored in a locked location, and each use must be recorded in a log. While such restrictions are burdensome in the laboratory, they can be prohibitive for fieldwork, especially if it entails international travel.

Barbiturates, given intramuscularly (IM), have also been used as anesthetics for reptiles (Wang et al. 1977; Miller and Gutzke 1998). Barbiturate anesthesia is associated, however, with variable dose requirements, long recovery times (up to 48 h), and occasional mortality (Wang et al. 1977; Arena et al. 1988; Miller and Gutzke 1998). Therefore, a barbiturate anesthetic is a poor choice for reptiles (Arena et al.1988). Interestingly, the effects of barbiturate anesthesia can vary seasonally (Köppl et al. 1990). Whether such variation applies to other anesthetics as well is not known. Barbiturates are listed as schedule II controlled substances by the DEA and thus have purchase and handling requirements similar to those of ketamine.

Propofol (5–10 mg/kg body weight) is becoming more popular as an injectable anesthetic; it is safe and has an extremely short induction time (Bennett et al.1998; Anderson et al. 2000), usually <3 min (Divers 1996). As with most injectable anesthetics, however, the dose response to propofol varies substantially among species (Anderson et al. 2000). Because its effects are brief (ca. 30 min), it is most appropriate for short procedures or as an induction agent for procedures conducted under gas anesthesia. The biggest drawback of propofol is that it must be given intravascularly or intraosseously, which makes its use in many reptiles difficult (e.g., most chelonians), if not impossible (e.g., small squamates). For chelonians, presedation with another anesthetic can aid in the administration of propofol (Pye and Carpenter 1998). In reptiles with vascular or intrarosseous access, propofol is likely the best injectable anesthetic for short procedures or anesthetic induction.

Alphaxalone in combination with alphadolone (in a 3:1 ratio; Saffan) given IM at a dose of 15 mg total steroid/kg body weight provides reliable and safe anesthesia for short (<20 min) surgical procedures in lizards and chelonians, but its effect is extremely variable in snakes (Lawrence and Jackson 1983). Recovery from alphaxalone/alphadolone anesthesia can take up to 4 hours, and in some animals violent twitching occurs during recovery (Lawrence and Jackson 1983). Other injectable anesthetic agents, such as tiletamine HCl/zolezapam HCl in combination (Telezol) and medetomidine (Domitor), may also be appropriate for reptiles in some circumstances (Stahl 1995; Norton et al. 1998; Sleeman and Gaynor 2000). However, published studies of these substances are limited, making it difficult to evaluate them. Because no single injectable anesthetic has proven consistently safe in all reptiles, an investigator should conduct preliminary trials on a small sample of individuals before employing an injectable anesthetic to many or valuable individuals.

HYPOTHERMIA

The effectiveness of low temperatures (e.g., immersion in wet ice) as an anesthetic is widely debated, particularly among members of Institutional Animal Care and Use Committees (see Martin 1995). Hypothermia provides exceptional immobilization, is simple to use, is extremely safe, and has been widely used as an anesthetic in small reptiles. It has a reasonable induction time, and animals quickly recover full activity upon the return to normal body temperatures (Calderwood 1971). Its analgesic properties are disputed, however, and cold narcosis may lead to brain necrosis (Northcutt and Butler 1974; Wang et al. 1977). Concurrent use of hypothermia and a local anesthetic (e.g., Xilocaine, 0.5%) is effective for minor procedures (e.g., implantation of a hormone pellet or PIT tag). In such instances, hypothermia serves predominantly as an immobilizing agent while another agent provides the appropriate anesthesia. Such a combination of treatments is especially useful in field studies involving small reptiles that need to be released immediately after manipulation. However, because the analgesic properties of hypothermia are not known, alternative agents with known anesthetic properties are preferable for most reptile procedures. Because anesthetic options available in some situations are limited, use of hypothermia should not be absolutely prohibited. Rather, an investigator should demonstrate why other agents cannot be used before selecting hypothermia as a means of anesthesia.

Anesthetic Support and Monitoring

Reptiles are very good anesthesia patients, as survivorship is typically 100 percent when investigators use an appropriate anesthesia regime effectively. However, one must not ignore the importance of monitoring a patient during anesthesia. Even with constant, carefully regulated anesthetic delivery, the depth of anesthesia can change over time, which can contribute to increased morbidity during prolonged procedures

(Barter et al. 2006). Thus, a discussion of anesthetic support and monitoring in reptiles is warranted.

VENTILATION

Reptiles are rarely ventilated during anesthesia unless they are anesthetized with a vaporizer. Even if forced ventilation is not used during gas anesthesia, its use during recovery can dramatically shorten recovery time. Isoflurane is not metabolized, so it is cleared from the body solely through the lungs. Anesthetized reptiles have a very low ventilatory rate, so clearance of isoflurane, and thus recovery, can be prolonged unless the animal is ventilated with anesthetic-free air. Periodic (ca. once every 2 min) forced ventilations through an endotracheal tube clear the lungs of accumulated isoflurane and, thus, hasten recovery. In chelonians, where intubation can be difficult, repeatedly pumping the forelimbs in and out of the shell opening enhances ventilation (Adest et al. 1988).

HEAT

Temperature profoundly affects physiological processes (Huey 1982), so, not surprisingly, maintaining an anesthetized reptile at or near its optimal temperature reduces induction and recovery time (Arena et al. 1988). Warming an anesthetized reptile above room temperature during surgery and/or recovery can substantially reduce total anesthesia time. Heat can be provided overhead (e.g., light bulb) or from below (e.g., water blanket or heating pad). However, since anesthetized reptiles clearly cannot thermoregulate behaviorally, investigators must use care to avoid hyperthermia. Monitoring cloacal temperature of the patient helps to ensure that appropriate body temperature is being maintained.

FLUIDS

Water balance in reptiles is critical, especially for those species that live in arid environments. Although the benefits of fluid therapy are difficult to quantify, supplemental fluids can minimize the negative effects of anesthetic and surgical procedures, especially among small reptiles. Fluids (e.g., reptile Ringer's Solution or saline at 10 ml/kg body weight) given subcutaneously or intracoelomically simply and safely replace fluids lost by evaporation due to exposure of internal viscera and blood loss.

ANESTHETIC MONITORING EQUIPMENT

Monitoring anesthetized reptiles facilitates early detection of anesthetic complications. Many means of monitoring are available (Murray 1996). Doppler ultrasound, for example, detects arterial blood flow and thus can be used to monitor heart rate. Pulse oximetry measures oxygen saturation of the blood and thus provides a valuable assessment of ventilation and gas exchange. Electrocardiography assesses the electrical conductivity of the heart and thus can be used as an indicator of cardiac function. In most instances, however, modifying monitoring equipment for use with reptiles can be difficult. For example, it is often difficult to find an anatomical location to which sensors con be applied in order to produce a continuous signal. Attempts to correct faulty or interrupted signals during a surgical procedure usually extend surgery times, which can be detrimental to the patient. Therefore, the decision to use anesthetic monitoring equipment should be based on the target species, the invasiveness of the manipulation, and the investigator's proficiency with the equipment. Monitoring equipment can be of value, but one must be competent at using the equipment, or it may present more problems than benefits.

Analgesics

Postsurgical analgesics can reduce or eliminate some of the typical signs associated with pain (e.g., reluctance to move, abdominal tucking, atypical aggression, depression, and failure to eat). Among reptiles, signs of pain are especially prevalent in chelonians that have undergone invasive procedures through their shells. While the assessment of pain in other reptiles can be quite difficult, one should assume that stimuli deemed noxious in other animals are likely noxious in reptiles.

Unfortunately, the pharmacokinetics and effectiveness of analgesic agents in reptiles have not been thoroughly studied (see Bennett 1998). As a result, analgesic agents are often not used in reptiles. Analgesics that have been used (Lawton 1999) include buprenorphine (Buprenex, 0.01 mg/kg body weight, IM), butorphenol (Torbugesic, 0.4–25 mg/kg IM, IV, or SC), carprofen (Rimadyl, 1–4 mg/kg IM, IV, SC, or PO), and flunixin meglumine (Banamine, 0.1–0.5 mg/kg IM).

Butorphenol is likely the most widely used analgesic in reptiles, although limited data in turtles suggest that even at a relatively high dose (28 mg/kg body weight), it may have little effect in reducing pain, and it can cause short-term respiratory depression (Sladky et al. 2007). In the same study, morphine (1.5 mg/kg) provided better antinociception but caused long-lasting (e.g., 8 h) respiratory depression.

The use of an analgesic agent should be based on the expected level of discomfort associated with the procedure being performed and the impact of the analgesic on normal function immediately postrecovery. Animals released into the wild shortly after manipulation may be at increased risk of predation or loss of social dominance (e.g., from decreased alertness, impaired coordination) if they are under the influence of a sedating analgesic agent.

Standard Data from Live Specimens

Patrick T. Gregory

Although some monitoring activities may involve sighting animals rather than capturing them, the latter method provides an opportunity to obtain much more information. Data on a range of parameters can be collected from a live reptile in hand. Most of those data can be taken "on the spot," allowing the animal to be released shortly after capture, and thereby minimizing its stress. However, some researchers prefer to take animals back to the laboratory for data collection and to release them later. The data recorded depend on the purposes of a study. If the main purpose is simply to monitor a population, then minimal data are required, although the number of variables measured may increase over time as questions multiply. Given the ease with which many measurements can be taken in the field and their broad potential utility, it is worthwhile, once an animal is in hand, to collect data on a basic set of variables. The following summary of those variables is based on my own methods, discussions with other researchers, and the literature.

Methods for capturing and handling reptiles are covered elsewhere in this volume (see above and Chapter 5, "Finding and Capturing Reptiles") and in other reviews (e.g., Plummer 1979; Fitch 1987). Large and/or venomous animals are particularly problematic, and special techniques are required to prevent stress or injury to either the researcher or the animal while data are collected (e.g., see Freed and Freed 1983; Galbraith and Brooks 1983; Gillingham et al. 1983; King and Duvall 1984; Gregory et al. 1989; Walczak 1991; Jones and Hayes-Odum 1994; Rivas et al. 1995). Restraint of the animal may facilitate time-consuming measurements or delicate procedures even for small individuals and harmless species (Mauldin and Engeman 1999).

Circumstances of Capture

It is generally advisable to record the circumstances of capture and behavior of individual animals (e.g., basking in open, under cover, in trap), as well as date, time of day, weather conditions, and features of the habitat, both in general and at the site of capture. Details such as shedding condition (e.g., blue eyes in snakes) should be noted (S. J. Arnold, pers. comm.), as should observations of behavior, including defensive behavior, prior to and upon capture and handling. Researchers should also keep records of animals seen but not captured, and their behavior, if possible.

Individual Identification

Almost any study will benefit from individual identification of animals. Investigators can avoid duplicate counts, determine growth and movements of recaptures, and estimate population size and survivorship (see Chapter 15, "Population Size and Demographics"). Individuals can be identified or recognized by numerous means (see Chapter 9, "Marking Reptiles"). The oldest, simplest ways to do this are to attach a physical marker of some sort to the animal or to clip or scar some part of its external anatomy. Deficiencies of these approaches include potential loss of tags or injury to the animal. In addition, some scars may eventually disappear or be confounded with later, natural wounds. The least invasive way to identify individual animals is with natural markings or color patterns, which can be recorded with drawings or photographs. Both recording patterns initially and checking through files of them for recaptures can be time consuming, but this method is useful even as an adjunct to other marking methods. Most researchers also describe injuries, missing or regenerating tail tips, missing limbs, scars, and scale anomalies, which aid identification (S. J. Arnold, pers. comm.; R. J. Brooks, pers. comm.), as well as meeting other objectives. I recommend that workers record as full a description as possible of an animal's distinctive features, regardless of any other marking procedure that is used. In some cases, photographs of every individual may be warranted.

Size

A measure that holds much potential information (e.g., individual identity, index of age and/or reproductive output) and that always should be recorded is an animal's size (Seigel and Ford 1988). Although body mass is easy to measure (at least for smaller species) and an important index of size, it usually is inadequate by itself. This is largely because body mass can vary significantly over time independently of other indices of body size, depending on factors such as food intake, level of activity, reproductive investment, and state of hydration. Thus, body mass is best considered in conjunction with some linear measure of body size. Together, the two can yield an estimate of an animal's relative mass or body "condition," which may allow an investigator to make inferences about its health or reproductive state (e.g., Forsman and Lindell 1996; Naulleau and Bonnet 1996); nevertheless, an investigator should exercise caution when interpreting such measures (Weatherhead and Brown 1996; Green 2001). In some lizards, tail width also may provide a reliable index of body fat and, hence, of condition (Doughty and Shine 1997).

The most common linear measure of body size is body length. In turtles, both carapace length and plastron length are used as standard indices of body size (Dunham and Gibbons 1990). Those two measures are correlated, although the relationships between them may differ between the sexes (Dunham and Gibbons 1990). Carapace length is usually measured with calipers as straight-line distance (Miller 1978; R. J. Brooks, pers. comm.). In large marine turtles, however, curved carapace length often is recorded instead, using a flexible tape measure (Dunham and Gibbons 1990). Dunham and Gibbons (1990) discussed the various ways in which linear dimensions are measured in turtles. The minimal standard measures that seem appropriate are straight-line length of both carapace and plastron, measured along the midline. If comparisons are to be made with data from other studies, however, other measurements (e.g., maximum carapace length) may also be taken (Dunham and Gibbons 1990; R. J. Brooks, pers. comm.). Because different measures are sometimes used, an investigator should always note with the data, the type of measurement he or she took.

In other reptiles, the two most common measures of body size are *snout-vent length* (SVL) and *total length* (TL). (Investigators must also record the definition of any abbreviations they use to avoid confusion; for example, TL=total length vs. TL=tail length.) SVL is the distance from the tip of the snout to the anterior edge of the cloacal opening and, thus, is the length of the head and body proper. TL is the length of the head, body, and tail combined. The difference between SVL and TL is tail length, which also can be measured separately. In crocodilians, which are difficult and dangerous to handle, it may be possible to estimate head size, as an index of overall size, from photographs (Gorzula 1984; Stewart 1988), but I focus here on measurements taken from captured animals. The literature on crocodilians is somewhat inconsistent, with most recent studies presenting measurements of SVL (e.g., Thorbjarnarson 1993; Allsteadt and Lang 1995; Congdon et al. 1995; Tucker and Limpus 1997; Verdade and Sarkis 1998; Da Silveira and Magnusson 1999), but a few using TL (e.g., Rootes and Chabreck 1993a; Thorbjarnarson 1996). In line with most common practice, SVL should be used as the standard measure of body size, with TL or tail length also recorded whenever possible. However, different measures or indices of body size of crocodilians may vary in precision and differ in accuracy between experienced and inexperienced workers (Zweig et al. 2004).

In squamates, SVL is the most widely used measure of body size (e.g., Parker and Plummer 1987; Gregory and Larsen 1993; Doughty and Shine 1997; Castilla and Bauwens 2000) and the key standard value to record from live specimens (Fitch 1987; Seigel and Ford 1988; R. Shine, pers. comm.). It is especially important to use SVL rather than TL in this taxon because many lizards exhibit tail autotomy (the practice whereby an animal detaches part of it tail as a defense mechanism to elude a predator; the animal may later regenerate the lost part; Arnold 1988), and some species of snakes frequently lose parts of their tails (e.g., Willis et al. 1982; Mendelson 1992). However, tail length (measured either directly, or indirectly via TL) may be important for particular objectives (e.g., testing factors influencing mating success of male snakes, Shine, Olsson, et al. 1999) and is therefore worth recording as well (Seigel and Ford 1988). One problem in measuring the SVL of snakes is that a snake's body is highly elastic; consequently, estimates of SVL differ, depending on the method of measurement used (Fitch 1987; Blouin-Demers 2003; Bertram and Larsen 2004; Setser 2007; Rivas et al. 2008; R. Shine, pers. comm.; pers. observ.). The most common and simplest way to measure SVL on a live snake is to stretch the snake along a meterstick or tape measure (Fitch 1987). One virtue of this method is that, unlike most others, it can be done with easily carried equipment (e.g. a folding ruler) at the snake's capture site. With practice, an investigator can obtain quite repeatable measurements and, if careful, not especially stress most snakes (pers. observ.). However, some species may be significantly stressed by this procedure and their subsequent growth negatively affected (Fitch 1987). Furthermore, the practice can be dangerous for the researcher if the snake is venomous, and difficult or even not feasible for strong or very large snakes (see "Handling Live Reptiles," above). Two or more people are required for measuring very large snakes, which are measured along the midline of the body with string (which itself is measured later) or with a tape measure, preferably the former (Rivas et al. 2008). Perhaps a more easily measured proxy, such as tail length, along with previously calculated predictive equations, could be used to estimate SVL of large or dangerous snakes.

Alternatives to holding and stretching a conscious snake include photographing it against a scale indicator (Hudnall 1982), anesthetizing it before measurement (Setser 2007), and using a squeeze box, which restrains the snake between soft foam and a piece of Plexiglas (Quinn and Jones 1974; Cross 2000; Bertram and Larsen 2004; Hampton and Haertle 2009). In the latter method, one draws a line, tracing the snake's body curves, on the clear Plexiglas top (or bottom in some variations) of the box and then measures SVL and TL from the length of the line, using a piece of string, a map measurer, or a flexible tape measure (Quinn and Jones 1974; Bertram and Larsen 2004; Hampton and Haertle 2009). In a variant of the squeeze-box method, the investigator uses a photocopier or portable scanner to obtain an image of the snake and then measures the snake's length from the image (Mao et al. 2009). Measures of SVL obtained with a squeeze box are significantly smaller than those obtained by stretching the same snake along a meterstick (Bertram and Larsen 2004; Setser 2007). Similarly, measurements of dead snakes, live and alert snakes, and anesthetized snakes differ (Fitch 1987; Blouin-Demers 2003; Setser 2007; R. Shine, pers. comm.), as measurements made by different people often do (Setser 2007; R. Shine, pers. comm.; pers. observ.). The relative repeatability of measures obtained with these various methods is generally unknown, as are their proportional relationships (but see comparisons of methods by Blouin-Demers 2003; Bertram and Larsen, 2004), although Setser (2007) found that measurements from anesthetized snakes were the most precise. The key issue here is consistency within a study (e.g., measurements all taken the same way by the same experienced person). If multiple people are taking measurements, results obtained by each of them from the same subset of the animals should be compared; correction factors can be calculated and applied to all measurements of individuals whose results consistently differ from those of other members of the team despite additional practice. Consistency between studies by different researchers is more difficult to achieve, but it is perhaps less important for monitoring and inventory projects than is within-study consistency.

Measuring lizards, which are neither as elastic nor flexible as snakes, does not present the same problems. However, small lizards are often difficult to hold for measurement, and prolonged handling increases the likelihood of autotomy in species that shed the tail. In such cases, a small squeeze box can prove very useful by immobilizing the lizard against soft foam, which does not harm the lizard but does reduce tail loss (P. Rutherford, pers. comm.). Alternatively, small lizards can be wetted and held by surface tension against the side of a plastic bag, allowing accurate and repeatable measurements (Wapstra 2005).

Various other morphometric measurements of reptiles (e.g., head length and width) are less broadly useful variables for most inventory or monitoring studies. However, they may be required to meet particular study objectives. Furthermore, for species in which measuring SVL is especially difficult, other morphometric measures, such as head length, can be used as proxies (e.g. Houston and Shine 1994b).

Age, Sex, and Reproductive Condition

Data on age are difficult to obtain but worth collecting when possible (e.g., to improve estimates of population size; Manly et al. 2003). The direct way to do this is to capture animals at birth and then to mark and release them. This procedure is time consuming, and its utility depends on good recapture success. Indirect methods of estimating age are more common. Age in some species of turtles has been estimated based on external annuli on the laminae of the shell (Graham 1979; Dunham and Gibbons 1990; St. Clair et al. 1994). This method estimates only minimum age in older turtles (Graham 1979; R. J. Brooks, pers. comm.). Annuli in claws that can be sampled from live animals may be a feasible way of determining age in some species of turtles (Thomas et al. 1997). In large turtles, age potentially can be determined via skeletochronology (see "Skeletochronology," in Chapter 10) on bone obtained in biopsies (Klinger et al. 1997). Skeletochronology has been used to determine age based on toes clipped when marking lizards (e.g., Pilorge and Castanet 1981) and on tail tips of snakes (Waye and Gregory 1998; Waye 1999). Obviously, this method is applicable to snake species that are subject to frequent natural loss of the terminal part of the tail, but not to those with specialized tails used for very specific purposes (e.g., rattlesnakes). In any case, it also is important to first establish that there actually is a relationship between age and the index used (Dunham and Gibbons 1990; Waye and Gregory 1998). These methods are discussed in detail in Chapter 10, "Determining Age, Sex, and Reproductive Condition."

Removal of tail tips or toes for age determination also provides tissue samples that can be stored for later genetic work.

Other tissues such as blood also can be sampled fairly easily from many reptiles (Cuadrado et al. 2003; ASIH 2004), and doing so routinely would provide a genetic library for both intra- and interpopulation studies (R. J. Brooks, pers. comm.).

Researchers always should record the sex of individuals caught, because it is a key element of population structure that influences population dynamics; sex is an additional character by which individuals can be identified. In many cases, sex determination is possible only for adults. Turtles often exhibit sexually dimorphic features that vary among taxa (Graham 1979), and the sex of crocodilians can be determined by cloacal examination (Rootes and Chabreck 1993a; Nuñez Otaño et al., 2010). Although many species of lizards and snakes exhibit sexual dimorphism, sex can usually be determined by testing for the presence or absence of hemipenes using probes (Fitch 1987) or hemipenal eversion, the latter working especially well for small specimens, including immature animals (Gregory 1983; Harlow 1996; also see Box 5, "Hemipenis Preparation," in Chapter 7). Fiber-optic laparoscopy has proven effective for large lizards whose sex is otherwise difficult to determine (Davis and Phillips 1991). In many species of snakes, sex can be determined, with experience, simply from the shape and length of the tail (Fitch 1987; pers. observ.). For a detailed discussion of these methods, see Chapter 10, "Determining Age, Sex, and Reproductive Condition."

It is important to report the reproductive condition of adult female reptiles. Especially when coupled with year-to-year recapture histories of individual females, this information allows estimation of reproductive frequency, which influences population dynamics. In turtles, the presence of eggs can be detected by palpation of the abdomen anterior to the hind legs or by X-ray photography (Gibbons 1990); the latter also allows the researcher to count eggs. Presumably, the X-ray approach could be applied to other taxa, although it requires the animal to be removed from the field. Ultrasound imaging has been used in a similar manner on crocodilians (Tucker and Limpus 1997). Gravid females of both lizards and snakes are recognizable by the distended condition of their abdomens, and eggs often can be felt by external palpation, especially in snakes. Because the eggs in snakes are arranged linearly within the body, one can often count the number of eggs by palpation (Fitch 1987; Farr and Gregory 1991).

Diet

In live snakes, especially larger individuals, it is relatively easy to palpate the abdomen and detect the presence of one or more food items (Fitch 1987; pers. observ.). In some cases, it is even possible to gently move the food forward to the mouth for identification, after which the food either can be removed for preservation or gently palpated back to the snake's stomach (Gregory 1984b; Fitch 1987). This is potentially stressful for the snake, however, especially if food items are large (Fitch 1987; pers. observ.). I do not recommend that fieldworkers use the procedure during inventory or monitoring programs unless actual identity of the prey is crucial to some aspect of the study. For most other kinds of reptiles, it is not possible to determine the presence of stomach contents or the type of food contained in live animals without invasive stomach-flushing or stomach-washing methods (e.g. Pietruszka 1981; Rowe 1992; Rivas et al. 1996; Rice et al. 2005), which are stressful and potentially fatal, especially for small species (e.g. Watters 2008). Again, investigators should do this only if necessary for a particular project. On the other hand, fecal samples can be taken relatively easily from some species by gently massaging the posterior part of the abdomen (Fitch 1987), so this is a feasible way to identify food items in those animals.

To summarize, the standard data that researchers should collect from live animals include the following: details of the capture (e.g., behavior, time, weather, habitat); color, pattern, other identifying features such as scars or injuries, as well as any mark given to the animal by the researcher; body mass, body length (usually SVL, except in turtles), tail length (except perhaps in turtles); age, where feasible; tissue samples, where feasible, for later genetic work; sex, reproductive condition of adult females (and possibly the number of embryos in snakes). Other variables should be recorded as objectives dictate.

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CHAPTER NINE

Marking Reptiles

MICHAEL V. PLUMMER and JOHN W. FERNER

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Introduction

Why is marking animals useful for biodiversity studies? Although basic inventories provide presence and absence data for a site, whether or not a species persists there depends on the status of the group or population. Analyses of mark-recapture data can provide population information (e.g., on abundance, survivorship, population trends; see Chapter 15, as well as "Permanent Plots," in Chapter 13) that enhances inventory and monitoring data. Likewise, radiotelemetry data can be directly relevant to biodiversity studies, contributing information on habitat preferences and estimating population size and survivorship (see methodology in White and Garrott 1990).

A number of authors have provided comprehensive reviews of techniques used to mark reptiles (e.g., Woodbury 1956; Spellerberg and Prestt 1978; Swingland 1978; Plummer 1979; Fitch 1987; Balazs 1999; Ferner 2007). We recommend that readers consult these publications for an in-depth view of available techniques. In this section, we focus on the techniques that are most commonly used with reptiles as well as on some of the more promising newer techniques. We have attempted to present material sufficient to allow a researcher to implement a technique without consulting original sources. However, we encourage potential users of complex technologies such as radiotelemetry, PIT tagging, and following genetic markers (FitzSimmons et al. 1999) to consult the original literature and confer with investigators experienced in using those methods.

Balazs (1999, pp. 101–102) described successful techniques of marking as "partly science, partly art, and partly guesswork." Ideally, marks should not interfere with an animal's behavior, growth, survival, or probability of recapture. They should be simple and easily applied in a field setting, be physiologically inert, and provide positive, accurate identification to the original researcher who marked the animals, any field assistants, and, especially in the case of long-lived species, future workers. In practice, one or more of these goals may be compromised by the size limitations posed by small species or juveniles of larger species. Marks can be group specific (e.g., age cohort, sex) or individual specific depending on the purpose of the study.

Marks fall into the following general categories: (1) identifying marks already on the animal (e.g., natural color pattern, scars) that can be photographed and subsequently recognized, (2) permanent or temporary tags attached externally, (3) morphological modifications (e.g., toe clipping), (4) color marking with paint or other comparable materials, and (5) telemetric devices. Various factors, both general ones and those specifically applicable to the reptilian group under investigation, should be considered when choosing an appropriate technique. In general, the mass of attachments or implants should not exceed 5 percent of body mass. Another important consideration is animal longevity, which in reptiles ranges from about a year in species experiencing almost complete annual population turnover (e.g., some small lizards) to decades in routinely long-lived turtles. Marks that can be applied to small juveniles and remain readable on large adults years later are unusual. Body shape, which varies considerable among reptile groups, is also important. Tags or transmitters appropriate for box-shaped turtles are unlikely to be suitable for slender, elongate snakes and many lizards. Finally, habitat preferences, behaviors, and other natural history traits can influence the choice of mark type; for example, many species of reptiles burrow in loose soils or sediments, which precludes attachment of external tags or transmitters.

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Identifying Marks and Photographs

The use of patterns or distinguishing marks to identify reptiles is not as common as it is in other groups of vertebrates. McDonald et al. (1996) and McDonald and Dutton (1996) found that the appearance (size, shape, color shade, and pattern) of the pink spot on the top of the head of adult Leatherback Sea Turtles (*Dermochelys coriacea*) was unique for each individual and a highly reliable means of identification. Nevertheless, identification of recaptures with these marks was less than 100 percent accurate, so they used the marks to supplement, rather than replace, flipper tags. It may be that the surface details of turtle scutes, such as patterns of grooves and ridges, will prove adequate for individual recognition. These patterns can be recorded in photographs, photocopies, graphite rubbings, and dental cast molds, which can be taken into the field for subsequent comparisons and identifications (Galbraith and Brooks 1987a).

Exuvia of captive snakes can be saved, and scars or irregularities noted to aid in identification of individuals (Henley 1981). Shine et al. (1988) successfully recognized individual snakes using the variable subcaudal scute formulae along with sex and body size.

In a field study, Stamps (1973) found that she could recognize individuals in a small group of *Anolis aeneus* lizards by their distinctive patterns coupled with various stages of tail regeneration. Carlström and Edelstam (1946) reported the successful use of black-and-white photographs to record unique individual dorsal patterns in *Lacerta vivipara* and throat patterns in *Anguis fragilis.* Because the repeated recaptures required by some marking techniques disrupted natural movement patterns, Rodda et al. (1988) identified individual adult *Iguana iguana* based on general coloration and the natural idiosyncrasies of the scales (e.g., variation in length, attitude, curvature, and tip type) of their dorsal crests. More recently, Sacchi and his colleagues (2010) demonstrated with two species of lizard (*Podarcis muralis* and *Lacerta bilineata*) that recaptured individuals could be identified with close to 100 percent accuracy (98% of recaptures within and 99% percent of captures between years), based on *lepidosis,* or characteristics (e.g., size and shape) and arrangement of scales. The investigators photographed scales in selected areas when the lizards were first captured and digitized them. Digitized photographs of recaptures were matched with photographs on file, using Interactive Individual Identification Systems (I^{3}S, Classic ver. 2.0) software (see Appendix II). The system works with individuals of any age (juvenile, subadult, adult), independent of color pattern, ornamentation, or lack thereof, and may be applicable to other reptiles, as well.

Permanent and Temporary Tags

A permanent tag is intended to remain on an animal throughout its life or at least for the length of the study, whereas a temporary tag can be as short lived as a few hours or days. Loss is known to occur at various rates for all types of attached or implanted tags. Tag loss should be minimized, but also recorded and included in analyses that assume no loss, such as mark-recapture analyses.

Many different types of external and internal tags, including plastic, metal, string-like spaghetti tags, beads, buttons, spangles, wire, foil, tape, bird bands, aluminum rings, luminescent glass tubes, bells, collars, and rivets, have been used on reptiles with various degrees of success. One of the most promising recent developments in permanent tagging is the *passive integrated transponder* (PIT) tag, a radio-frequency device that transmits a unique numeric or alphanumeric code when inductively powered by a reader placed within a few cm of the tag. A PIT tag allows identification without having to capture and handle the animal. The biologically inert, reusable, glass-encapsulated tag contains no battery (hence the term "passive") and has an estimated life span of up to 75 y. The most common frequency currently used for reptiles is 125 kHz. PIT tags circumvent identification problems sometimes encountered when using morphological modifications by providing a positively identifiable code that is readable by anyone. The main disadvantages of PIT tags are the high costs of both tags (ca. US$5 ea.) and reader (ca. US$1,500), a high rate of tag loss, and in some cases, movement of tags. In addition, PIT tags are inappropriate for studies that rely on tag reportage from the general public. Tag injection sites should be chosen to provide practical scanner access and to minimize chances of tag breakage from normal locomotor movements and collisions.

Radioisotopes have been used as tags to mark and track small turtles, lizards, and snakes (Ferner 2007). However, radioisotope techniques are not commonly used in reptiles because of limited detection distance, inability to discriminate among individuals, potential danger to study animals, high cost, legal restrictions on isotope use, and the recent development of more effective remote sensing techniques such as miniature radiotransmitters and PIT tags.

Tagging Different Reptile Groups

Turtles

Various types of metal and plastic tags have been attached to hard-shelled turtles (Plummer 1979; Ferner 2007). The preferred site is the posterior carapace, to which tags can be attached with screws, glue, or wires running through holes drilled in the shell. Tags can be numbered or colored differently for remote identification (Loncke and Obbard 1977; Buhlmann and Vaughan 1991). Metal tags attached to *Chelydra serpentina* provided identification from up to 40 m through a telescope and remained in place for at least 3 years with no loss (Loncke and Obbard 1977). Because hatchling turtles have relatively soft shells, attaching a tag can pose a problem. Layfield et al. (1988) found that metal wire rings passed through holes punched in the posterior marginal scutes in hatchling turtles had high retention rates. Internal wire tags inserted into the flipper of hatchling or larger turtles can be detected by X-ray equipment or magnetized for later detection by a magnetometer (Balazs 1999). Some tags pose a hazard to turtles, as they tend to get caught on fish nets and discarded fishing line (Graham 1986). Metal and plastic tags are placed on the proximal trailing edge of a front flipper of sea turtles despite low retention rates in all species (Balazs 1999). Tag material affects tag life and retention rates. *Dermochelys* on St. Croix, for example, retained only 16 percent of monel (nickel-copper alloy) tags beyond 4 years, but tags made of other materials were lost much sooner (McDonald and Dutton 1996). Compared to monel, titanium is less affected by corrosion, but unfortunately it is colonized by barnacles, which increases drag, tearing of tissues, and eventual tag loss (Parmenter 1993). Bellini et al. (2001) found that inconel (a nickel-iron-chromium alloy) tags had a much higher retention rate during a 5-year study of *Eretmochelys imbricata*. Balazs (1999) provided an excellent practical guide to tagging sea turtles.

Tags should be loosely attached to the carapace of softshells because of the tendency of the fleshy shell to become necrotic at the point of any firm attachment of a foreign object. Cattle ear tags have been attached to the rear carapacial edge in many hardshelled species by first drilling a hole in one of the marginal scutes. For *Carettochelys,* which lacks marginal scutes, the hole must be drilled into the suture line between the marginal bones to avoid necrosis and subsequent tag loss. Tags so attached remain in place for at least 4 years (S. Doody, pers. comm.).

Although internal PIT tags have been used in relatively few freshwater (Buhlmann and Tuberville 1998) and sea (Fontaine et al., 1987; Parmenter 1993; McDonald and Dutton 1996; Balazs 1999) turtles, we highly recommend them, both for ecological studies and for tracking commercial use. PIT tags can be especially practical for tagging Leatherback Sea Turtles, which lose external tags at an extremely high rate (Balazs 1999). Retention of PIT tags appears to be relatively high in all species on which they have been used. Apparent tag loss from large-size species may actually reflect an inability to detect the tag because the tag and reader are too far apart or because individuals doing the reading have not been properly trained (McDonald and Dutton 1996). Buhlmann and Tuberville (1998) recommended the use of PIT tags on small freshwater turtles but not on hatchlings. Although recommendation of standard PIT tag implantation sites may be premature, the body cavity in the anterior inguinal region parallel to the bridge of the shell appears to be a good site for freshwater turtles (Buhlmann and Tuberville 1998), and subcutaneous or intramuscular locations in the shoulder appear appropriate for sea turtles. Tags in these regions provide reliable readings and move very little (Parmenter 1993; McDonald and Dutton 1996). PIT tags should not be implanted intraperitoneally in species of turtles that get to be large, because tag movement in the body cavity can place the tag beyond the detection distance of the reader. We do not recommend placing tags in predrilled holes in the shell, because of relatively high rates of shell breakage and tag loss.

A variety of devices have been temporarily attached to the posterior portion of the carapace of turtles to provide short-term spatial and behavioral information. Thread trailing involves following a continuous thread that is pulled from a spool attached to the carapace or from a trailing device pulled behind the turtle (Reagan 1974; Wilson 1994; Claussen et al. 1997). To locate nest sites of female *Chrysemys picta,* J. Congdon (pers. comm.) temporarily attached transmitters to their posterior carapaces with duct tape. Darkened tin foil has been molded to the back of hatchling *Malaclemys terrapin* for redetection with a metal detector inside artificial field enclosures (A. Tucker, unpubl. data). In comparatively obstruction-free waters, stiff lines or wires can be attached to the posterior carapacial edge to tow highly visible, streamlined flotation tags. Such tags should be fabricated with a "weak link" so that they will detach from the carapace easily if they become entangled. Sea turtles in the open ocean may tow helium-filled balloons to increase long-distance visibility obscured by the curvature of the earth.

Lizards

A variety of materials have been used for external tags of lizards. Tags generally are easier to "read" in the field than morphological modifications (e.g., toe clipping) and can be used in conjunction with more permanent markings. Minnich and Shoemaker (1970) marked *Dipsosaurus dorsalis* with colored Mystik cloth tape that they placed in various color combinations of bands around the base of the tail. Deavers (1972) tagged *Uma notata* with small pieces of foil attached to a 30-cm piece of light string, which was tied around the lower abdomen. The tag allowed him to measure the burial depth of the lizards in the sand at night. Similarly, Judd (1975) attached a square piece of aluminum foil, 5 cm on a side, to a 1-m length of red thread to locate buried *Holbrookia propinqua* for body temperature readings.

Rao and Rajabai (1972) tagged agamid lizards (*Sitana ponticeriana* and *Calotes nemoricola*) with colored aluminum rings of various sizes. These rings were placed around the thigh and caused no apparent problem. In a field study of *Uma inornata,* Fisher and Muth (1989) sewed a series of small (2.0 × 2.5 mm) plastic jewelry beads to the base of the tail with surgical steel monofilament. Henderson (1974) tagged *Iguana iguana* by tying small "jingle bells" around their necks with fishing line! Zwickel and Allison (1983) applied pressure sensitive rip-stop nylon tape to the backs of *Emoia physicae* after washing the skin with 95-percent alcohol. The 5- × 10-mm pieces of tape were then color-coded with acrylic paint. Colored plastic bird bands glued to the tails of *Aspidoscelis sexlineata* lasted much longer than field marks painted on the tail (Paulissen 1986).

Clark and Gillingham (1984) made nocturnal observations of *Anolis* sp. by gluing small capillary tubes filled with a phospholuminescent liquid to the lizard's dorsum at dusk. The glowing markers, visible up to 30 m away, lasted up to 6 hours and were sloughed off by the lizards within 24 h with no apparent harm.

Snakes

External tags (e.g., jaw tags, plastic buttons, beads, spangles) have been used on snakes only rarely (Spellerberg and Prestt 1978; Fitch 1987; Ferner 2007); we do not recommend their use, because of the potential problems of entanglement in vegetation and limited access to narrow burrows for an otherwise streamlined body.

Although PIT tags show promise as an ideal marking system for snakes (Camper and Dixon 1988; Keck 1994b; Jemison et al. 1995), tag movement and low retention rate may be of concern. Deep intraperitoneal rather than superficial subcutaneous injection may reduce tag loss (Jemison et al. 1995). All tags that Roark and Dorcas (2000) injected intraperitoneally in the mid-body region of subadult *Pantherophis guttatus* were retained, whereas more than 50 percent of the tags injected in the neck region moved posteriorly and were expelled in the feces. PIT tags can be successfully introduced to snakes via implanted prey, but retention by the snakes from ingestion to defecation may be only 2 weeks (MacGregor and Reinert 2001).

Crocodilians

PIT tags have been used successfully to mark hatchling caimans (Dixon and Yanosky 1993), although their use on juvenile or adult crocodilians has not been extensively evaluated. Tags were injected into the base of the tail just behind the insertion of the hind limb. PIT tags should not be injected intraperitoneally, because of possible migration in the body cavity, which, in these large animals, could move the tag out of detection range of the reader. Dixon and Yanosky (1993) regarded PIT tagging as superior to most other systems of marking caimans.

The large projecting scutes of crocodilians provide plenty of opportunities for attaching tags. Bayliss (1987), for example, inserted harpoon tags into the neck scutes of subadult and adult Australian crocodilians. These numbered tags, large enough to be read from a distance, were held in place with harpoon-like barbs. Tags have also been attached to foot webbing. These large reptiles have also been fitted with neck collars, which are commonly used to mark large mammals (Brandt 1991).

Morphological Modifications

Modifying the morphology of the study animal is an inexpensive and rapid method of marking large numbers of animals in the field. However, application techniques of individual researchers and healing patterns of individual animals vary, so positive identification may be difficult, especially for field technicians lacking experience with the system and the study species.

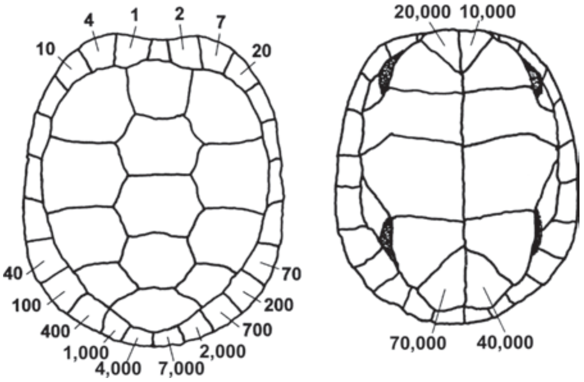

FIGURE 41 Ernst et al.'s (1974) numerical coding system for hardshelled turtles as exemplified by Pond Sliders (*Trachemys scripta*). (Left) Carapace with numerical code for each marginal scute. (Right) Plastron with numerical code for each gular and anal scute. A unique specimen number is obtained by adding the numerical values assigned to each notched scute. For example, *T. scripta* No. 1474 would have notches in marginal scutes nos. 1000, 400, 70, and 4. Modifications of this system may be necessary to accommodate turtles with only 11 marginals on a side (e.g., kinosternids).

Turtles

The hard upper shells of freshwater and terrestrial turtles have been subjected to the most kinds of marking techniques. The marginal scutes on the carapacial edge have been cut, sawed, filed, ground, and drilled in various coding schemes to provide unique identification marks. Unfortunately, the carapacial edge is also particularly subject to natural injury from a variety of sources (e.g., predators). Such injuries can obscure identification marks already present or serve as an additional identification mark, if inflicted before marking.

Cagle (1939) assigned a number to each marginal scute from anterior to posterior on each side of the carapace so that he could designate the scutes that he notched. He used a comma to separate marginals on the same side and a hyphen to separate the left and right sides. Thus, in turtle number 2,9-1,3, he marked marginals 2 and 9 on the left side and 1 and 3 on the right. With up to four notches, more than 2,000 different turtles can be numbered uniquely with Cagle's system. In a simpler system providing more available numbers, scutes are modified for sequential numbering (e.g., Ernst et al. 1974; Fig. 41). The marginals at the bridge or juncture of the carapace and plastron (usually the 4th–7th marginals) are not used by most researchers. However, bridge scutes can be used like any other scute, provided that marks are applied with a triangular file (A. Tucker, pers. comm.).

J. Congdon (pers. comm.) used a simple sequential system employing letters, rather than numbers, to mark more than 14,000 turtles of several species. He lettered the marginals from A to M (includes turtles with 13 marginals), anterior to posterior, on the turtle's right side (A–K in kinosternids) and N to Z on the left (N–X for kinosternids). Marginals D to F on the right and R to T on the left are not used. From 2 to 4 scales are notched and read sequentially (e.g., AB, CJ, HIJ, IKWX). This simplified scheme can be applied uniformly to turtles with different numbers of marginals; one only has to remember the alphabet and that the first marginal scale on the right is letter A and on the left, letter N. Cogdon consistently recognized the more than 40,000 individuals he recaptured over 26 y. This coding system also reduced the frequency with which new or part-time participants in a research project misread the identifications (J. Congdon, pers. comm.).

Softshell turtles (Trionychidae), lacking epidermal scutes and dermal bone at the carapacial edge, can be marked by removing small V-shaped pieces of carapace with a sharp knife or punching holes in the carapace with a paper punch (Doody and Tamplin 1992). The V-shaped or circular marks quickly fill out to the carapacial edge as healing progresses but persist as distinct whitish scars best seen from the ventral surface. Coding is more difficult because of the lack of discrete marking sites, that is, individual scutes. One softshell coding system involves numbering positions 1 through 12 as if a clock face were superimposed on a dorsal, posterior view of the carapace (Plummer 2008). Using a combination of from 1 to 5 carapacial cuts in combination with toe clips, Plummer (1977; unpubl. data) marked more than 1,000 *Apalone mutica* and *A. spinifera*.

Hatchling and young sea turtles have been group-marked by surgically exchanging small pieces of tissue between the carapace and plastron. These "living tag" tissue graphs produce contrasting pigment patterns that are retained by older turtles (Balazs 1999).

Lizards

Toe clipping is by far the technique most commonly used to mark lizards for identification. Tinkle (1967) developed a numbering system for *Uta stansburiana* that involved clipping up to four toes, but no more than two per foot and never adjacent ones. The removal of toes 4, 8, and 20, for example, would give the lizard a code number of 4-8-20 (Fig. 42). Medica et al. (1971) developed a different numbering system (Fig. 42) similar to the one often used for salamanders and frogs. In that system, at least one toe from each foot is cut to eliminate the problem of a lizard having lost a digit(s) accidentally being mistaken for a marked animal. Woodbury (1956) suggested lettering the feet (A–D), numbering the toes (1–5), and using combinations of those designations along with the sex of an individual for its identification code. A male with the first

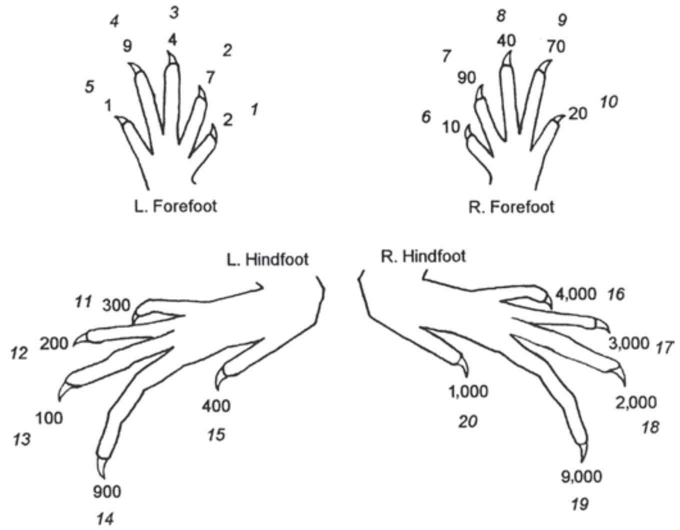

FIGURE 42 Dorsal views of lizards with numbered toes. The outer numbers (italicized) represent Tinkle's (1967) system for numbering lizard toes. The inner numbers represent Medica et al.'s (1971) system for numbering lizard toes. A unique specimen number is obtained by adding the numerical values assigned to each clipped toe.

toe of his left front foot and the second toe of his right hind foot clipped would be A1-D2-Male. In his alphanumeric code, Waichman (1992) also gave each foot a letter and numbered the toes on each limb 1 through 5. Using this code and clipping up to three toes, but no more than two per foot, 1,310 lizards can be individually marked.

Woodbury (1956) suggested that toe clipping might have a harmful effect on lizards, but this does not appear to be the case. Australian skinks loose toes naturally at a relatively high rate with no major effect on survivorship (Hudson 1996), which indicates that toe clipping may be an appropriate marking technique. Dodd (1993b) found that toe clipping had no immediate or long- term effects on sprint performance of *Aspidoscelis sexlineata* when only two toes were clipped per individual. Finally, the sprint speed of *Sceloporus merriami* was not correlated with the number (up to four) of toes clipped per individual (Huey et al. 1990).

Snakes

Early on snakes were marked with tattoos, hot brands, and freeze brands (Spellerberg and Prestt 1978; Fitch 1987; Ferner 2007), which are still used occasionally (e.g., Burns and Heatwole 1998). Nowadays, however, most researchers mark individual snakes by clipping either subcaudal or ventral scales. Brown and Parker (1976b) devised a simple and practical serially numbered system of clipping ventral scales just anterior to the vent (Fig. 43). Clipping ventral scales may be preferable to clipping subcaudals because ventrals are larger, easier to clip, and remain even if the tail breaks; clipping ventrals may, however, be more traumatic to the snake (Fitch 1987). Clipped scales, of whatever type, need to be periodically reclipped, as the clips often become obscured by regeneration after several years, which hinders indentification (Fitch 1987).

To clip ventrals, the tips of small, sharp-pointed scissors are inserted under the posterior edge of the scute to be marked, which is then cut in an anterior direction over its entire length,

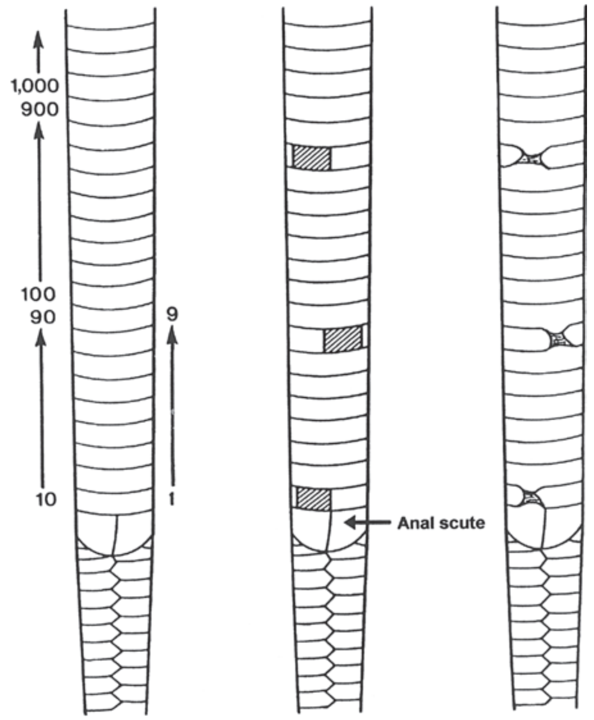

FIGURE 43 Ventral scale clipping system for marking snakes. Ventral view of posterior body of a North American Racer (*Coluber constrictor*). (A) Enumeration of ventrals proceeds anteriorly from the anal scute: series of scales indicating 10s, 100s, and 1,000s on the observer's left; scales indicating units on the observer's right. (B) Snake freshly marked with number 718. (C) Snake 718 three years after initial marking, showing appearance of scars. (From Brown and Parker 1976b; © *Jounal of Herpetology,* redrawn with permission.)

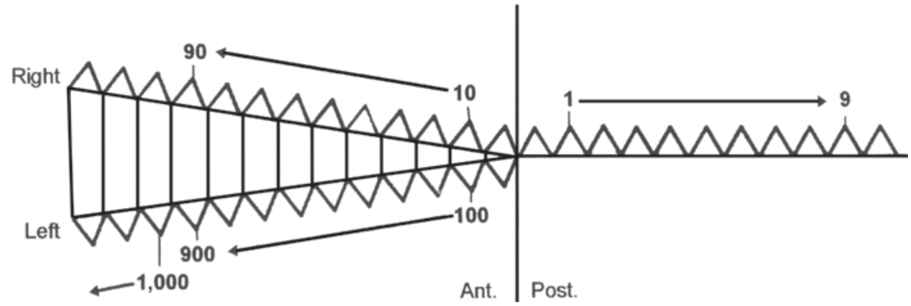

FIGURE 44 Simplified numbering scheme for marking crocodilians. The single row of enlarged dorsal median scales divides into two dorsolateral rows anteriorly. The diagram presents a lateral view of the posterior portion of the tail and a dorsal view more anteriorly. Immediately anterior and posterior to the division point are the "0" scales from which radiate the 1s, 10s, and 100s. Variations are used for alligators, caimans, and gavials (A. Tucker, pers. comm.).

back to front. Each mark requires two longitudinal cuts, one to the side and one toward the middle of the scale, roughly one-half the width of the scale apart. These cuts are then joined anteriorly with a transverse cut, and the isolated rectangular block of tissue is removed (Fig. 43). A small piece of scute must remain laterally so that the scute can be counted later, after healing. The cut also must be deep enough to remove the entire dermis; otherwise ,regeneration may obliterate the mark. In some snakes the ventral scalation may be anomalous, for example, with a scute duplicated on one side (e.g., Plummer 1980). Anomalous scute patterns and scute injuries must be incorporated into any scute-marking scheme.

Crocodilians

Various workers have notched or cut dorsal crest and tail scutes on crocodilians in several accepted marking schemes, but no systematic or standardized marking system has been published as has been done commonly for turtles, lizards, and snakes. This lack of standardization probably reflects the large number of scutes available for marking and the different marking needs of commercial farms, wildlife management agencies, and investigators carrying out population studies (A. Tucker, pers. comm.). A proposed scheme for marking dorsal scutes is shown in Figure 44.

Color Marking

A variety of temporary color marks (e.g., numbers, letters, color codes) have been applied to reptiles to facilitate visual recognition at a distance. Small amounts of quick-drying, nontoxic paints applied with a brush, spray can, or paint pen/marker work well and have been used on turtles (e.g., Auth 1975), lizards (e.g., Medica et al. 1971), snakes (e.g., Henderson and Winstel 1995), and crocodilians (e.g., Seebacher and Grigg 1997). Temporary paint marks for some species may be applied without capture (e.g., Passek and Collver 2001). Xylene-based paint pens should be avoided for most species; xylene is toxic and can affect behavior and survival (Boone and Larue 1999; Quinn et al. 2001). Because color marks are temporary, they are usually used in conjunction with more permanent techniques. In *Opheodrys,* paint marks began flaking off after 2 to 3 days and were completely gone after about 2 weeks (Plummer 1981). Similarly, underwater-curing, epoxy-paint marks on *Caretta* were visible for about 14 days (Tucker et al. 1996). Penney et al. (2001) successfully injected visible implant elastomer (VIE) to mark *Plestiodon reynoldsi.* Developed for marking migratory fish, the liquid elastomer is injected subcutaneously and hardens into a flexible mark within a few hours. In an extensive study of *Sceloporus jarrovii,* Simon and Bissinger (1983) found no differences in survivorship between animals with conspicuous or more cryptic color makings. Similarly, mortality did not differ significantly between individuals of *Sceloporus undulatus* marked with a spot of paint at the base of the tail and those left unmarked (Jones and Ferguson 1980).

Color marks on hard body parts persist the longest. The turtle carapace is an obvious place to paint identification numbers and letters. Paint should be applied sparingly to small turtles with rapidly growing unossified carapaces, because the paint may hinder growth and cause disfigurement. Although rattlesnake rattles have been variously marked with tags, beads, disks, spangles, and so forth, color marking has become more or less standard practice. Color marking allows for quick recognition of marked animals at a distance and also provides data on shedding frequency (Brown et al. 1984). Paint can last up to about 8 y on individual rattle segments of *Crotalus horridus* (W. S. Brown, pers. comm.).

An unusual form of short-term color marking is the use of powdered fluorescent pigments. The fine powder readily adheres to a reptile's body, and as the animal moves through its habitat, it leaves powder traces that can be detected with a portable UV light. This technique has been used to track small lizards and turtles (e.g., Fellers and Drost 1989; Butler and Graham 1993; Stark and Fox 2000), and in combination with thread trailing, greatly facilitated locating tortoise nests (Keller 1993).

Radiotelemetry

Radio-frequency transmitters are usually used in conjunction with more permanent marking techniques such as scute marks or PIT tags. With the notable exception of sea turtles, few reptiles are large and mobile enough to require triangulation or satellite telemetry. Indeed, a common problem in reptilian telemetry is how to pinpoint the location of small animals at very close range. Using a receiver with an "attenuator function," which maintains signal directionality at very close ranges, greatly facilitates this task. Microprocessor dataloggers have been attached or implanted in various reptiles and are usually used in conjunction with radiotelemetry. A disadvantage of most dataloggers is that the logger must be recovered for data retrieval. The marine units used on sea turtles, however, are exceptions; they are capable of relaying data via satellite from a datalogger mounted on the turtle to a computer in a researcher's lab.

Transmitters and other telemetry equipment are available from a number of manufacturers that are usually listed with contact information on websites that compile links to biotelemetry information. Several such sites are listed in Appendix II. A visit to any of these sites quickly reveals an overwhelming number of biotelemetry uses and manufacturers and can easily confuse one not familiar with applications specific to reptiles. Although common elements exist among applications, telemetry systems, especially transmitters, usually are configured to meet each user's unique needs. This specificity is especially true for reptiles, which as a group vary greatly in morphology and habitat preferences. Researchers new to telemetric applications in reptiles should survey articles published in major herpetological journals (e.g., *Copeia, Herpetologica, Journal of Herpetology*) and identify sources of telemetry equipment. Various sources are also listed in Appendix II. In addition, we recommend that new users consult both the primary literature and established researchers who use telemetry on reptiles for accepted practices and advice on using this technique.

Turtles

Despite strong attenuation of radio-frequency signals under water, VHF radio-frequency transmitters have been commonly used on aquatic and marine, as well as terrestrial, turtles. Low-frequency (sonic) transmitters designed for fish transmit well under water and have been used on a variety of marine turtle species (Eckert 1995, 1999) but only on one freshwater turtle species (Moll and Legler 1971). The Argos satellite system, a worldwide tracking and environmental monitoring system, has been widely used to track marine turtles globally. Platform transmitter terminals, mounted on turtles, transmit various kinds of data (e.g., latitude and longitude, temperature, date, time, number and duration of dives) to an orbiting satellite (Renaud et al. 1993; Renaud 1995; Beavers and Cassano 1996), which picks up the signals and relays them in real-time to over 50 ground stations located at points around the globe. Data are then sent to one of two Argos centers, which processes the data and delivers the information directly to Argos users' desktops around the world.

Boarman, Goodlett et al. (1998) reviewed the methods of transmitter attachment in 113 radiotracking studies of turtles. They grouped attachment methods into six categories: (1) adhesives (e.g., dental acrylic, epoxy, silicone sealant), (2) harnesses, (3) wire, screws, bolts, cable, nylon ties, or monofilament line passed through holes drilled in the carapace, (4) surgical implantation, (5) sewing, and (6) taping. Transmitters should be flat and closely conform to the carapace to prevent entanglement in filamentous algae or other vegetation and should be placed away from the peak of the carapace to prevent obstruction of narrow or low burrows.

Transmitters should be loosely attached to the fleshy, highly vascular shells of softshells (Trionychidae) to minimize shell necrosis and subsequent transmitter loss. Plummer and Burnley (1997) and Plummer et al. (1997) attached transmitters to the posterior carapace of softshells with a single stainless-steel wire punched through the carapace and held loosely in place on the underside with a plastic button. The cigar-shaped transmitter was loose enough to pivot freely. Both transmitters and dataloggers have been implanted into the body cavities of softshells (Plummer and Burnley 1997; MVP, unpubl. data).

Transmitter trailing devices consisting of a trailing transmitter alone (J. Demuth, unpubl. data) or a transmitter in combination with a spool of thread (Lemkau 1970) and a datalogger (Plummer 2003) have been commonly used on turtles of the genus *Terrapene*. Trailer packages should be streamlined and attached close to the carapace so as to pivot freely.

Lizards

Transmitter packages used in early telemetry studies of lizards, such as *Iguana iguana* (Montgomery et al. 1973) and *Sceloporus occidentalis* (McGinnis 1967), were considerably larger and heavier than those available today. However, attaching transmitters to the backs of small lizards still remains a challenge. Richmond (1998) fabricated a harness for small lizard species using rubber from a bicycle inner tube. The backpack harness used by Ussher (1999) on tuatara (*Sphenodon* sp.) held transmitters weighing less than 4 g. The harness consisted of a 30- × 20-mm pad of porous polypropylene webbing with two 4-mm-wide polyester straps and two lengths of 13-mm-wide polyester elastic braid. The straps attached the transmitter to the pad. The braid, one length passing around the neck of the lizard and the other around its body behind the forelimbs, attached the pad to the animal. The harnesses were reliable and lasted up to 5 months. They also proved safe for the lizard, if correctly fitted to minimize or prevent abrasion. Fisher and Muth (1995) made a similar backpack for attaching radio transmitters to *Phrynosoma mcallii,* using polypropylene tape and clear polyurethane elastic, which are available at most fabric stores. Small radio transmitters can also be surgically implanted into lizards. Wang and Adolph (1995) found that these implants did not alter the thermoregulatory patterns of free-ranging Western Fence Lizards (*Sceloporus occidentalis*).

Snakes

The refinement of miniature implantable transmitter packages has revolutionized ecological research on snakes in recent years (Shine and Bonnet 2000). Transmitters are usually surgically implanted intraperitoneally, or in larger-size species, subcutaneously; the antenna is implanted subcutaneously. Detailed descriptions of surgical techniques can be found in Reinert and Cundall (1982), Weatherhead and Anderka (1984), and Reinert (1992). Transmitter shape may not be critical in large or heavy-bodied species such as *Crotalus* and many boids, but in many colubrids, especially juveniles, transmitter size and shape may be limiting. Elongate, flattened transmitter packages are preferable to short, cylindrical designs. Most transmitter manufacturers will work with researchers to provide needed shapes. Some snake researchers purchase unpotted transmitters without batteries and shape the final package themselves. Temperature dataloggers have been similarly implanted in heavy-bodied *Crotalus* (M. Dorcas, unpubl. data). Researchers should avoid implanting transmitters late in the activity season because cool weather can hinder healing of the surgical wound (Rudolph et al. 1998). They should also monitor the effects of the surgery on snake behavior and physiology (Charland 1991; Lutterschmidt and Rayburn 1993).

Ciofi and Chelazzi (1991) sewed external transmitters to the backs of individual *Coluber constrictor flaviventris,* but their technique has not been used widely. Transmitters may be force-fed to snakes for short-term monitoring. The transmitter is eventually passed in the feces by normal peristaltic movements but will be retained if a restriction thread is tied around the body in back of the transmitter (Fitch 1987). However, we strongly recommend against the use of restriction bands to increase the time a transmitter is retained. Harlow and Shine (1988), Shine (1991a), and Steve Beaupre (pers. comm.) have enticed dangerously venomous elapids and viperids to voluntarily ingest mice containing transmitters, and Burns and Heatwole (1998) enticed the sea snake *Aipysurus laevis* to ingest fish containing sonic transmitters. However, having a transmitter in the digestive tract may alter normal snake behavior (Fitch 1987; Lutterschmidt and Reinert 1990).

Harmonic direction finders have been used to track two snake species (Webb and Shine 1997; Engelstoft et al. 1999). These novel devices are passive, like PIT tags, and thus do not require power sources. Consequently, they can be exceedingly small (0.5 mg). Harmonic direction finder technology holds promise for tracking snakes (Webb and Shine 1997; Engelstoft et al. 1999) and lizards too small for radio transmitters, whose lower size limit is determined by the battery. Current disadvantages of harmonic direction finders include short detection distance, lack of individual recognition, false signals, tags not available commercially, and the high cost of the transceiver (ca. US$6,000; Engelstoft et al. 1999).

Crocodilians

The large size of crocodilians and their projecting dorsal scutes provide various opportunities for implanting and attaching transmitters and dataloggers. Transmitters have been attached externally in a variety of locations, including on neck collars (Rootes and Chabreck 1993b) and on the dorsal caudal scutes (Muñoz and Thorbjarnarson 2000). For short-term tracking of location and body temperature, *Paleosuchus* and *Crocodylus porosus* can be induced to swallow prey carcasses with transmitters inside (Magnusson and Lima 1991; Grigg and Seebacher 2001). For additional information on the use of radiotransmitters with crocodilians, see “Radiotelemetry,” under “Swamp-Dwelling Crocodilians,” in Chapter 11.

Cautions and Recommendations

1. Shell notching, toe clipping, and scale clipping are reliable and inexpensive methods for marking reptiles. However, these techniques require skill, and investigators should practice them before employing them in the field.
2. Newer techniques for marking should be tested in the laboratory but then used in the field only in conjunction with proven methods until they are deemed accurate and reliable.
3. All tags to be applied externally should be tested for possible interference with locomotion, mating, and foraging. Tags that snag vegetation may be a problem for species inhabiting lush vegetation in both terrestrial and aquatic environments. We do not generally recommend external tags for snakes, burrowing or legless lizards, or amphisbaenians.
4. Color marking, used in addition to more permanent marks, can facilitate field identification and minimize the need for recapture.
5. External tags, transmitters, or color marks may make animals more conspicuous to predators and to conspecifics.
6. Tag loss is an important issue in mark-recapture studies because it violates a primary assumption of population estimation methods. Rates of tag loss should be recorded and included in analyses that assume no loss.
7. PIT tags can be an excellent technique for marking providing positive identifications for many species of reptiles. Before employing pit tags, however, investigators should carry out preliminary work on the species in question, especially snake species, to determine whether the tags will be retained at a useful rate and whether or not they will affect growth and subsequent population dynamics. PIT tags should not be injected into the body cavity of large individuals because of possible tag movement beyond the detection distance of the reader. Also, we do not recommend placing tags in pre-drilled holes in the turtle shell.
8. Telemetry is an extremely valuable tool for obtaining large amounts of data on relatively few individuals; however, it is not without problems. We caution new telemetry users that the “art” of finding animals in the field can be more difficult than might first appear. Thus, one should allow sufficient time to get past the learning stage before actual data collection begins.
9. Investigators should take precautions when employing marking techniques that require contact with blood or other body fluids to prevent the spread of disease among animals. Application tools should be disinfected after each animal is marked. Balazs (1999) recommended that investigators employ two sets of tagging equipment, one for healthy animals and another for obviously diseased animals.

Acknowledgments

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CHAPTER TEN

Determining Age, Sex, and Reproductive Condition

ROBERT N. REED and ANTON D. TUCKER

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Introduction

When first encountering an animal, an observer usually asks, "What is it?" Once basic species identification is achieved, the observer likely moves on to questions about the animal's sex, age, and reproductive status because such information is central to understanding the biology of any population of interest. In many reptiles, however, accurate determination of the sex, age, and reproductive status of an individual (especially a live one) may require taxon-specific techniques. In this chapter, we discuss standard methods for determining these basic attributes in reptiles.

Use of some of these methods requires substantial experience with and a "feel for" the organism. We therefore recommend that investigators take the time to examine live (if available) and preserved specimens of pertinent taxa before undertaking field research. We also recommend that investigators work with a suitable taxon expert in the field to learn the nuances and common pitfalls associated with each technique. Finally, validation studies are usually necessary to identify sources of error in assigning sex, age, and other attributes. For hard validation, it may be necessary to sacrifice a series of individuals to determine whether judgments based on external characters are borne out by internal anatomy.

We have organized the text within each attribute (i.e., sex, age, reproductive condition) section by taxonomic group. We thus discuss Lepidosauria (hereafter referred to as "snakes and lizards," and including tuataras, except where noted), Crocodylia (crocodilians), and Testudines (turtles) as more or less distinct entities within each of the primary attribute sections. Where similar methods apply to multiple taxa, we attempt to avoid repetition by referring the reader to the appropriate section.

Determining Sex

Snakes and Lizards

PRIMARY SEX CHARACTERISTICS

Male squamates (lizards and snakes) are distinguished by the presence of paired copulatory structures called *hemipenes* (Fig. 45). These inverted structures lie within chambers formed from invaginations of the tail that open into the cloaca. One hemipenis or the other is everted from the cloacal region of the male and into the cloaca of a female to achieve copulation. In many taxa, the presence of hemipenes is indicated by a thickened tail base or prominent paired ventrolateral bulges immediately posterior to the cloacal opening, which can be used to assign sex. Hemipenes of many squamate taxa can be manually everted by pressing the underside of the tail posterior to the vent (i.e., "popping" the hemipenes). This technique is especially effective for small lizards and juvenile snakes, but it is difficult to use on larger species, species with muscular tails, and fragile species. Eversion of the entire hemipenis is unnecessary for determining sex and may cause injury if done with excessive enthusiasm. Male tuataras lack copulatory organs.

Female snakes may have *caudal scent glands,* the papillae of which can be partially everted at the posterior margin of the cloaca and mistaken for hemipenes. Often these papillae can be differentiated from hemipenes by color, size, and shape, as scent glands are usually small, white, and somewhat pointed when everted, whereas hemipenes are large, vascularized, red or pinkish, and blunt when everted (Klauber 1956). Female squamates may also possess hemiclitori inverted into chambers in the tail; these hemiclitori may be everted when pressure is applied just posterior to the cloaca. These generally tiny organs may conceivably be mistaken for hemipenes in

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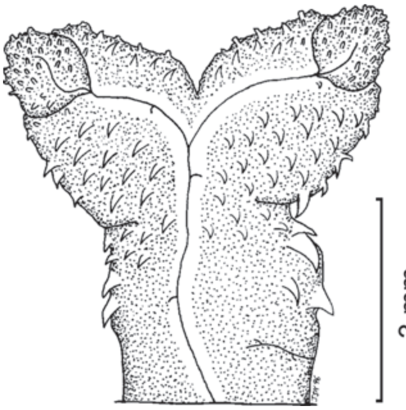

FIGURE 45 A single hemipenis of the Australian elapid snake *Furina ornata*, illustrating the bilobed appearance and complex morphology of many squamate hemipenes. (Drawing by J. S. Keogh from Keogh 1999. *Zoological Journal of the Linnean Society* 125: 239–278. Wiley-Blackwell Publisher; reprinted with permission.)

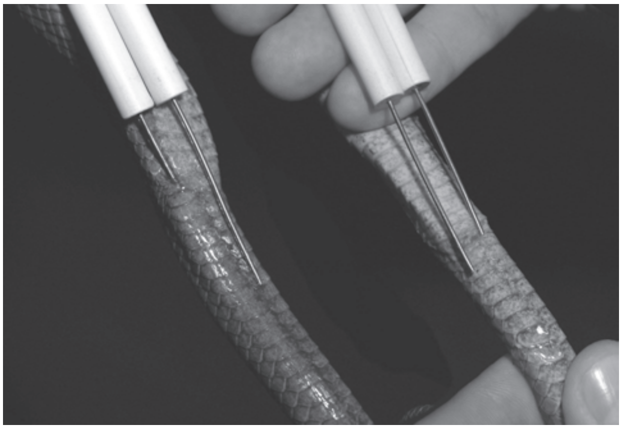

FIGURE 46 Probing snakes to determine sex. Male (left) and female (right) Brown Treesnakes (*Boiga irregularis*); each has a probe inserted posteriad from the cloaca, with a second probe lined up with it externally to illustrate the insertion depth. The male snake probes to approximately eight caudal scales deep; the female probes to approximately two caudal scales deep. (Photo courtesy of S. R. Siers.)

groups such as the monitor lizards (family Varanidae), in which the *hemiclitori* are large and erectile (Böhme 1995).

When manual eversion of the hemipenis is not possible, sex often can be determined by sliding a blunt probe distally from the posterior end of the cloaca near the vent and just off either side of the ventral midline—probes typically travel farther into the tail of a male, because the male tail contains inverted hemipenes (Fig. 46). In contrast, probes typically travel a shorter distance into the tail of a female before running into a tissue barrier that represents the distal end of a homologous, but much smaller, invagination in the female tail (Blanchard and Finster 1933; Schaefer 1934). On the other hand, hemipenis length varies among taxa, as do strength of surrounding muscle and depth of homologous structures in females. In addition, Schildger and Wicker (1989) showed that the depths of hemipenile invaginations in lizards of six genera (*Trachydosaurus, Egernia, Corucia, Tiliqua, Heloderma,* and *Varanus*) do not differ between the sexes.

In most snakes and some lizards, the progress of the probe's ball-tip can be observed as it moves into the tail. Once the probe has reached its maximal penetration, one can count the number of subcaudal scales corresponding to the maximal depth. In most snake species, subcaudal counts for males and females show little or no overlap (Fig. 46). Once determined for a series of individuals of both sexes within a species, the number of subcaudal scales corresponding to probe depth can be a fast and reliable metric for sex determination.

If a probe is inserted too far anteriad into the cloaca before probing posteriad, it is possible to insert the probe into the small *anal sacs* present in some species. Among some large-bodied iguanid lizards, however, sex can be determined by inserting a probe into the *cloacal pouch* (just anterior to the cloaca) rather than into the inverted hemipenes. In *Iguana* and *Amblyrhynchus,* for example, the cloacal pouch is deeper and wider in males than in females (Dellinger and von Hegel 1990; Rivas and Ávila 1996).

While probing sounds easy, it can be difficult in practice. Lubricating probes with surgical lubricant or petroleum jelly greatly eases the process. Ball-tipped metal probes of various sizes for probing squamates are available in sets. Probes should always be sterilized between uses to minimize risk of pathogen transfer between animals. Other implements can be used as probes, if their ends are blunt enough to avoid damage to hemipenes or caudal tissues. Fitch (1987), for example, used clean grass stems to probe thousands of Kansas snakes. The stems had soft, pliable tips that could not injure internal tissues and were readily available in the field. Straightened plastic-tipped bobby pins make ideal probes, as they are disposable after a single use and thus minimize risk of pathogen transfer (DeNardo 1996). Narrow probes are required for species with narrow hemipenile cavities and must be used with great care to avoid puncturing the distal end of the hemipenis or puncturing tail tissue in females, which can result in misidentification of sex. Brown Treesnakes (*Boiga irregularis*), for example, have narrow hemipenile cavities, and plastic-covered flexible telephone wire can be use to probe the snake without causing damage (G. H. Rodda, pers. comm.). If an investigator suspects that she has punctured tissue with a probe, then she should always probe the other side of the cloaca as well. Males typically probe to an equal depth on each side, whereas a female with punctured tissue on one side should probe to a shallower depth on the other side.

Hemipenes can also be everted by injection of saline (for live animals) or formalin (for sacrificed specimens to be preserved) into the base of the tail (Nickerson 1970). For especially small or delicate individuals, eversion by sterile saline injection may be safer and more reliable than manual eversion or probing, but the advantages can be easily nullified by overaggressive injection and resultant tissue damage. Large reptiles may have to be anesthetized before hydrostatic eversion to overcome the strength of hemipenis retractor muscles (DeNardo 1996).

Several additional techniques for determining the presence of hemipenes are available. Ultrasonography has been used to determine sex in some lizard species (*Varanus albigularis, V. komodoensis, Heloderma horridum, H. suspectum*; Wright et al. 1995; Morris and Alberts 1996; Morris et al. 1996). The technique is highly accurate for these lizards, except for individuals with excessive intestinal gas. Laparoscopy has been used to determine sex in a few reptile species. In this procedure (largely synonymous with endoscopy), a laparoscope (flexible or rigid lighted tube) is inserted into the body cavity via a lateral incision in the skin and body wall to view the gonads (Schildger and Wicker 1989; Cree et al. 1991). A laparoscope can also be inserted through the cloaca into a caudal chamber to determine whether an inverted hemipenis is present (Davis and Phillips 1991).

Radiography has been used to sex lizards of a few species. The hemipenes of adults in many *Varanus* spp. include ossified structures (*hemibacula*) that can be visualized by radiography, although they are not visible in juveniles (Shea and Reddacliff 1986; Card and Kluge 1995). The width and length of the ischial bones of helodermatid lizards can be determined from radiographs and tend to be relatively larger in females than in males (Card and Mehaffey 1994).

Less technologically intensive solutions may suffice for some taxa. Davis and Leavitt (2007) reported that Desert Night Lizards (*Xantusia vigilis*) and several other lizard species could be sexed accurately by "candling." The investigator holds a lizard ventral side up and directs a narrow flashlight beam through the tail from the dorsum. If the animal is a male, both of the hemipenes should be visible. This method is ideal for small, moderately pigmented lizards, small snakes, and juveniles of larger species.

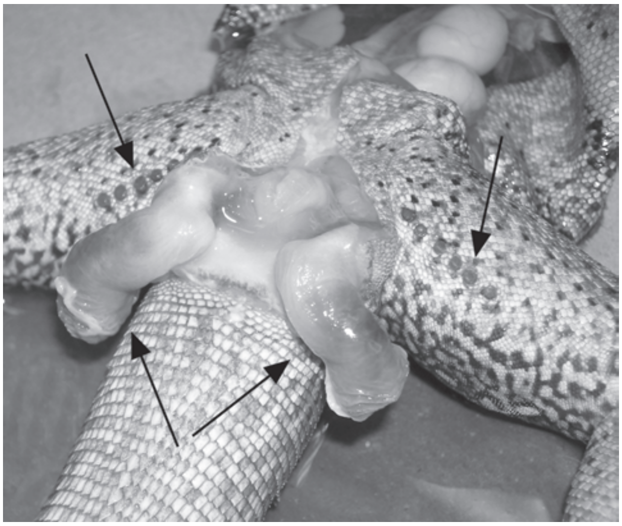

FIGURE 47 Ventral view of a male *Ctenosaura similis* during necropsy, illustrating the paired hemipenes (lower arrows) and the row of six enlarged femoral pores (upper arrows) on each hind leg. (Photo courtesy of Eric A. Tillman, U.S. Department of Agriculture.)

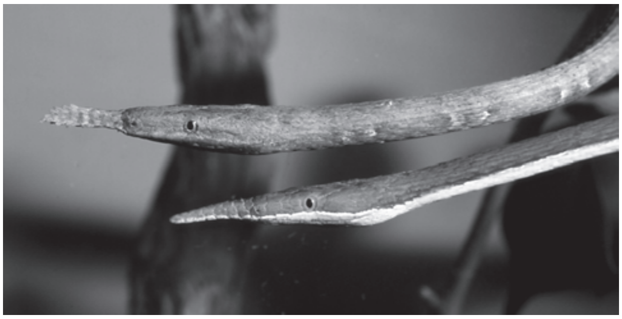

FIGURE 48 Female (top) and male (bottom) *Langaha nasuta* from Madagascar, illustrating sexual dimorphism in color pattern and rostral projections. (Photo courtesy of K. Krysko.)

SECONDARY SEX CHARACTERS

The number, position, and morphology of dermal scales often vary by sex within a species. For example, in many lizard taxa the femoral pores of adult males are larger and/or more numerous than those of adult females, although male pore size may vary seasonally (Rivas and Ávila 1996; Fig. 47). There are also sex-specific scale characters. Males of many phrynosomatids, polychrotids, and relatives have an enlarged pair of post-cloacal scales, whereas males of some varanid (Gaulke 1997) and eublepharid (D. DeNardo, pers. comm.) lizards have spiny scales lateral to the cloaca. Male-specific scale characters in snakes include chin pits or tubercles and/or keeled scales around the cloaca (Shine 1993). Boas and pythons possess cloacal spurs, or vestigial remnants of hind legs; typically (but not always), they are larger in males than in females (DeNardo 1996). Although the trends vary among species, male snakes typically have longer tails than females and, thus, more subcaudal scales, whereas females may have longer bodies or greater girth and, thus, more ventrals and/or mid-body scale rows. Male lizards may also have more extensive head ornamentation (e.g., *Pogona, Basiliscus*), larger dorsal crests (e.g., *Anolis* [= *Ctenonotus*] *cristatellus, Sphenodon*), or larger dewlaps (e.g., *Iguana*) than females. Bizarre differences occur in a few snake species, such as the Madagascan Leaf-nosed Snake (*Langaha nasuta*), in which the rostral projections are spinose in males and foliose in females (Fig. 48). Investigators familiar with these types of secondary characteristics can often use them to determine the sex of adults from a distance, precluding the need to capture the animal.

Sexual dimorphism in color and pattern, although widespread in squamates, is most common in some groups of lizards (especially Iguania and Scincomorpha; reviewed in Cooper and Greenberg 1992). In most sexually dichromatic species, males are more brightly colored than are females (Fig. 49). The differences in color, such as the bright orange heads of male Broad-headed Skinks (*Plestiodon laticeps*) during the breeding season as opposed to the brown head of females, may be visually obvious at a distance (Cooper and Vitt 1988) or hidden, as are the brightly colored ventral throat and/or belly patches in many phrynosomatid lizards and the extensible dewlaps in polychrotids (Cooper and Greenberg 1992). Sexual dichromatism is considerably less common in snakes, but it does occur in several species (e.g., *Vipera berus, Crotalus lepidus klauberi*; Shine 1993). In some squamates, sex-specific color changes occur seasonally or on even shorter time scales. Female crotaphytid lizards, for example, develop orange or red lateral blotches during the breeding season (Stebbins 1985), whereas male San Pedro Side-blotched Lizards (*Uta palmeri*) may develop darker dorsal colors and brilliant blue spots after aggressive territorial encounters (D. Hews, pers. comm.).

Many species of snakes and lizards are characterized by extreme sexual dimorphism in body size. However, the sex that attains the larger size varies by species (Shine 1993). Once the direction and degree of sexual size dimorphism in a population are known, sex can often be assigned to some individuals simply because they are larger than the largest members of the opposite sex; such is the case with the extremely large females of *Python reticulatus* and very large males of *Varanus komodoensis* and *Sphenodon*. The degree of sexual size dimorphism may vary within a species, however, as is the case with geographically disjunct populations of the North-western Carpet Python (*Morelia spilota variegata*) in Australia. Females in some populations are less than 10 percent longer than males, whereas those

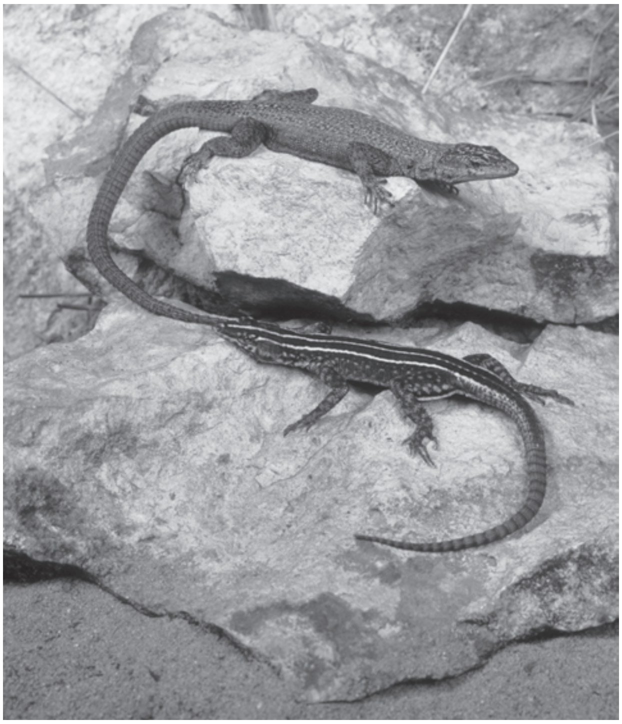

FIGURE 49 Male (top) and female (bottom) Common Flat Lizards (*Platysaurus intermedius*), illustrating sexual dimorphism in secondary sex characteristics such as color pattern. In life, the dimorphism is even more pronounced, as males are bright green and females are brown. (Photo courtesy of L. W. Porras.)

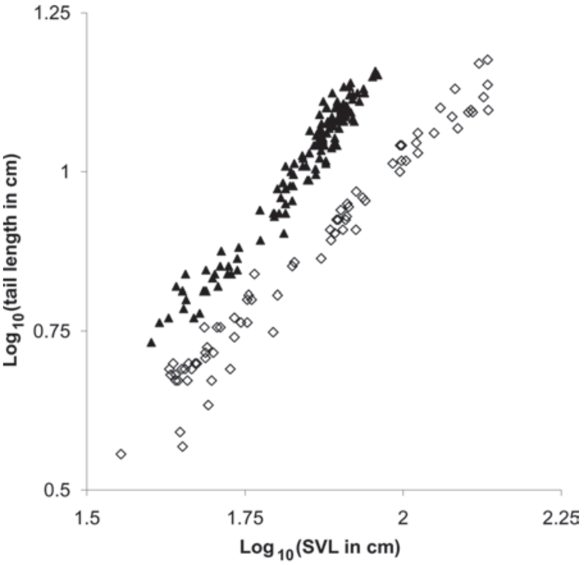

FIGURE 50 Tail length as a function of snout-vent length in a population of *Laticauda colubrina* (Serpentes, Elapidae) in the Republic of Vanuatu (both axes measured in cm and $\log_{10}$-transformed). Tails of males are longer than those of females of the same SVL, so sex can generally be assigned to individuals based solely on body size measurements and knowledge of the relationship between these two variables at the population level. (R. N. Reed, R. Shine, and S. Shetty, unpubl. data.).

in other populations average more than twice the length of males (Pearson et al. 2002).

Body proportions of males and females can also differ markedly, sometimes allowing for accurate assignment of sex. Male lizards often have larger heads than females, although this relationship is reversed in flying lizards of the genus *Draco* (Shine, Keogh, et al. 1998) and in many snakes (Shine 1991b). Adult male *Sphenodon* tend to have larger heads yet slimmer body profiles than females, as well as more prominent dorsal spines with wider bases (N. Nelson, pers. comm.). Male snakes and many male lizards have longer tails than do females of the same snout-vent length (Klauber 1943; King 1989; Shine, Olsson et al. 1999). Sex can thus be reliably assigned in some species by measuring these two variables, calculating their ratio, and comparing the ratio to that of other individuals from the same population (Fig. 50). Relative tail length is also useful for determining sex in preserved specimens for which dissection is undesirable, although some authors have noted that snout-vent length shrinks in preservative relatively more than does tail length (Fitch 1987; Reed 2001).

TEMPERATURE-DEPENDENT SEX DETERMINATION (TSD)

In many reptiles the sex of an individual is not determined genetically but by incubation temperature during the middle third of embryonic development (Bull and Vogt 1979; Wibbels et al. 1994). For most squamates with TSD, high temperatures at this time produce males, and low temperatures produce females (Janzen and Paukstis 1991). For species for which pivotal sex-determining temperatures are known, sex can sometimes be predicted if incubation temperatures are known. However, the utility of TSD for determining the sex of an animal in hand in the field is limited. For additional discussion of TSD, see “Turtles,” below.

KARYOTYPY

Tuataras do not appear to exhibit chromosomal sexual heteromorphism (Wylie et al. 1968), although this trait is known from a number of squamate lineages. Heteromorphy appears to be a derived condition in snakes; basal lineages such as the Boidae are generally homomorphic, with the degree of heteromorphy increasing in more derived lineages such as Colubridae and Viperidae (Bull 1980). Heteromorphy appears to have arisen independently within several lizard lineages, most notably in Iguanidae (*sensu lato*) and Pygopodidae (King 1977; Bull 1980). However, many squamate taxa have not yet been karyotyped, and our knowledge of the presence and degree of heteromorphy has not been surveyed for more than 30 years (Bull 1980).

PARTHENOGENESIS

Parthenogenesis has arisen multiple times within squamate lineages, usually as a result of interspecific hybridizations. Such hybridogenic events have resulted in numerous all-female species capable of reproducing without fertilization by a male, although copulation may be required (Darevsky et al. 1985). Parthenogenetic species occur primarily within the families Lacertidae, Teiidae, and Gekkonidae (Vrijenhoeck et al. 1989). Only one parthenogenetic snake (*Ramphotyphlops braminus*) is known; however, several suspected parthenogenetic events have been observed in individual snakes of other spe-

TABLE 13

Techniques for Diagnosing Reproductive Status of Reptiles[a]

Technique	*Cost*	*Portability*	*Training Required*	*Detects*	*Disadvantages*
Dissection	Low	High	Low	All stages	Sacrifice of animal
Surgery	Low	High	High	All stages	Low processing rate, not always possible in the field
Radio Immuno-Assay (RIA)	High	Low	High	Hormone levels	Not a field diagnosis
Palpation or cloacal examination	None	High	Low	Shelled eggs	Qualitative information only
Laparoscopy	Low	High	High	All stages	Qualitative information only
X-ray	High	Limited	Moderate	Shelled eggs	Does not detect follicles
Magnetic Resonance Imaging (MRI)	Very high	Low	High	All stages	Not a field diagnosis
Computerized Axial Tomography (CAT scan)	Very high	Low	High	All stages	Not a field diagnosis
Ultrasound	Moderate	High	Moderate	All stages	Limited image resolution

a. Adapted from Tucker and Limpus (1997).

cies (Schuett et al. 1997). The sex of individuals of known parthenogenetic taxa is rarely in question. However, individuals that appear to be males based on morphology occasionally pop up in species that are generally considered to be parthenogenetic, including *Ramphotyphlops braminus* and *Lepidodactylus lugubris*.

Turtles

Sexing an adult turtle is simplified if secondary sexual characteristics are present (Ernst and Barbour 1989). Males have a larger, thicker, and wider tail than females, and the cloaca is closer to the tail tip than to the plastral margin; in females, the cloaca is closer to the plastral margin. Some (but not all) turtles exhibit sexual differences in plastral morphology; in males the plastron is commonly concave, whereas in females it is flat to slightly convex (e.g., *Testudo, Terrapene*). The flexibility and shape of the posterior plastral margin in females of some species (*Chelodina longicollis, Podocnemis erythrocephala*) facilitate egg laying. Male tortoises that engage in combat may have gular plates that protrude farther anteriorly than those of females (*Kinixys erosa, Geochelone yniphora*). Some males have more vividly colored heads and necks during the breeding season than do females (e.g., *Batagur baska, Callagur borneoensis*), as well as different shapes of carapacial markings (e.g., blotch patterns are said to be sex specific in trionychids), eye colors (e.g., *Terrapene carolina, Clemmys guttata*), size dimorphisms (e.g., *Graptemys, Carettochelys*), nail elongation in adult males (e.g., *Terrapene, Trachemys scripta, Caretta caretta*), and melanism among older males (*Trachemys scripta*). Patches of rough skin that provide traction during mating are found on the rear surfaces of the thighs in males of a few species (*Sternotherus odoratus, Acanthochelys pallidipectoris*). Chin glands that are used for scent marking are more prominent in male than in female *Gopherus* and in some of the Platysternidae and Emydidae.

A few species of turtles (e.g., *Pseudemydura umbrina*) and juveniles of most other species of turtles lack external sex-specific characters, so sexing them is problematic. Generally, nondestructive methods of sexing (Table 13), including laparoscopy, ultrasonography, and hormonal assays of blood samples (discussed under "Snakes and Lizards," in the section on "Determination of Reproductive Condition," below) can be used. Sex can be confirmed by direct observation of the gonads when specimens are sacrificed or preserved. Dissections of adults should be conducted with reference diagrams depicting the appropriate species in hand (Ashley 1962; Rainey 1981; Wyneken 2001). Sex of hatchlings can be determined by direct observation of the gonads *in situ* or histological study of the gonads (Merchant-Larios 1999). Turtles can be sexed from blood or tissue samples using karyotypes, H-Y antigen (an antigen that induces the embryonic gonad to develop into a testis) or serum testosterone assays, or Bkm-probe DNA fingerprinting. Karyotypes are of limited use for detecting heteromorphic sex chromosomes in species that lack TSD. H-Y antigens are sex-specific in many vertebrates and proposed as a marker for sex; however, the procedure for finding these antigens is labor intensive and not readily applicable to large sample sizes. DNA fingerprinting using the Bkm probe has revealed sex-specific bands on chromosomes of some vertebrates and has been applied to *Lepidochelys kempii* and *Chelonia mydas* (Demas et al. 1990).

Lance et al. (1992) reported a hormonal approach to determining sex of hatchling turtles by using FSH to stimulate testosterone production. Serum or plasma testosterone levels can be measured by radioimmunoassay (RIA) to determine sex in numerous turtle species (Bolten et al. 1992; Owens 1999; Wibbels 1999), although plasma testosterone levels fluctuate in both males and females. Wibbels et al. (1990) noted several problems associated with these techniques. He was able to avoid many of them, however, with use of the proper protocols. For example, if investigators take blood samples immediately after capturing an animal, they can avoid possible stress effects on its testosterone levels. Plasma testosterone levels proved highly accurate (98% correct) in assigning sex to immature Desert Tortoises (*Gopherus agassizii*) >50 mm in carapace length, based on a small volume of plasma (50–100µl; Rostal et al. 1994). The challenges are finding a suitable blood vessel and technique for sampling blood (Owens and Ruiz 1980; Jacobson et al. 1992; Kuchling 1999). Blood samples can be obtained from venipuncture of the cardiac muscle, orbital sinus, jugular vein, carotid artery, scapular vein, brachial vein or artery, tail veins, or dorsal cervical vein. In general, smaller species offer fewer sites appropriate for venipuncture.

In all Cryptodira turtles except the Trionychidae, sex determination (TSD) is temperature dependent; in the Pleurodirea, in contrast, sex determination is genetic (Valenzuela and Lance 2004). Pivotal temperatures that determine sex in TSD species can vary both among species and geographically within a single species. Furthermore, the temperature experienced by an individual egg may depend upon its position in the egg chamber. The integration of varying environmental temperatures throughout the crucial middle trimester of development is not fully understood, but incubation temperatures can be monitored in the wild with dataloggers or controlled during incubation experiments in the laboratory. Thermal profiles can be used to predict offspring sex ratios with reasonable accuracy. When entire clutches are projected to be comprised of a single sex, individuals can be assigned that sex with some confidence as long as the model chosen best represents the incubation period. Choices include a model of constant temperature equivalents (Georges 1997) or a simpler logistic regression model (Mrosovsky et al. 1999).

Crocodilians

Crocodilians are sexed by determining if a penis is present inside the cloaca. After restraining an animal safely on its back, the investigator inserts a finger into the cloaca and palpates anteriorly for the penis, a protruding structure in medium-size or larger animals. The female clitoris is not easily felt. Hatchlings and small juveniles are sexed by squeezing the base of the cloacal mound or gently spreading apart the vent with blunt-tipped forceps to view the sex organ. Webb et al. (1984) reported that the tip of the genitalia in male hatchling *Crocodylus porosus* and *C. johnsoni* is wide and bulbous, whereas in female hatchlings is it relatively narrow and tapered. This method should be applicable in a broad range of species, given the conservative anatomical design of crocodilians (Richardson et al. 2002).

Determining Age

Snakes and Lizards

The most reliable method of knowing the age of free-ranging individuals is by marking and releasing neonates and subsequently recapturing them. This can be accomplished only by diligent long-term research at a given study site. Given the dearth of long-term studies of snakes and lizards generally, such unequivocal age designations are rare. Under these circumstances, investigators have used various methods to estimate ages of turtles and crocodilians.

SKELETOCHRONOLOGY

Skeletochronology is the science (or art) of determining the ages of individuals by examining their bones. It depends on periodic (usually seasonal) differences in rates of bone deposition. Under microscopic examination, cross sections (i.e., transverse sections) of limb bones appear banded. Periods of active bone growth are reflected in wide zones of bone deposition; narrow annuli bordering such zones reflect periods of slowed or arrested bone growth and are typically referred to as *lines of arrested growth,* or *LAGs* (Castanet et al. 1993; cf. Zug and Rand 1987, who use slightly different terms).

Much of the literature on skeletochronology in reptiles and amphibians is based on nonlethal sampling of phalangeal (toe) bones. Because the bones sampled are typically small, a number of steps are required before LAGs can be viewed. Most of these steps are standard in histological preparation of tissues prior to microscopic examination, including cleaning, decalcification, and dehydration of the bone, sectioning with a microtome after being embedded in paraffin, and staining using a tissue-appropriate stain. Although hematoxylin is the stain used traditionally, other researchers have used toluidine blue (e.g., Waye and Gregory 1998) and Jordet Dip-Quick Stains (Rossell and Sheehan 1998). Hasumi and Watanabe (2007) described staining tiny amphibian bones with acid fuchsine to make them easier to see and handle during paraffin embedding; after embedding, the fuchsine is washed out prior to final staining. Rossell and Sheehan (1998) compared the cost and efficacy of two histological staining techniques for skeletochronology in phalangeal bone from a salamander; these stains should have similar effects on reptile bones.

Caudal (tail) vertebrae are sometimes used instead of phalangeal bones (especially among limbless reptiles) in skeletochronological analyses and typically undergo similar histological preparation. Comparative studies indicate, however, that jawbones (mandibles and ectopterygoids) and femurs are more reliable for inferring age than either phalanges or caudal vertebrae (e.g., Castanet and Naulleau 1985; Castanet et al. 1988), but the former require lethal sampling. Different bones from the same animal may yield different results; in *Sphenodon,* for example, growth annuli remain discernible on femurs longer than on phalanges (up to 35 y in the former versus 20–25 y in the latter; Castanet et al. 1988). Waye and Gregory (1998) used caudal vertebrae to estimate the ages of gartersnakes (*Thamnophis* spp.), but the method was labor intensive, and results from the vertebrae were not highly correlated with results from jawbones (ectopterygoid) in a validation study. Minakami (1977, 1979) used somatic vertebrae from vipers (*Trimeresurus*), and Collins and Rodda (1994) used mandibles from Brown Treesnakes (*Boiga irregularis*) to estimate the ages of their study animals. In both of these validation studies, captive snakes were injected with a colored bone marker (lead-acetate, calcein, or alizarin red) to enhance visibility of LAGs. Erickson (2003) used external growth rings visible under a dissecting microscope in the glenoid cavities of vertebrae from known-age Bengal Monitors (*Varanus bengalensis*). His results demonstrated a strong correlation between the growth rings and age in years, but visualizing all growth rings required careful removal of calcified cartilage using a dental pick and rotary brush. Larger bones such as mandibles can be photographed under a dissecting microscope, eliminating the need for paraffin embedding and sectioning and, thus, decreasing preparation time and chemical costs (e.g., Collins and Rodda 1994; Fig. 51).

In species that inhabit temperate climates, skeletochronology is assumed to reflect annual patterns of growth resulting from discrete annual activity cycles. This assumption may be of dubious validity for species in the tropics, where activity may be less strongly seasonal. Collins and Rodda (1994) demon-

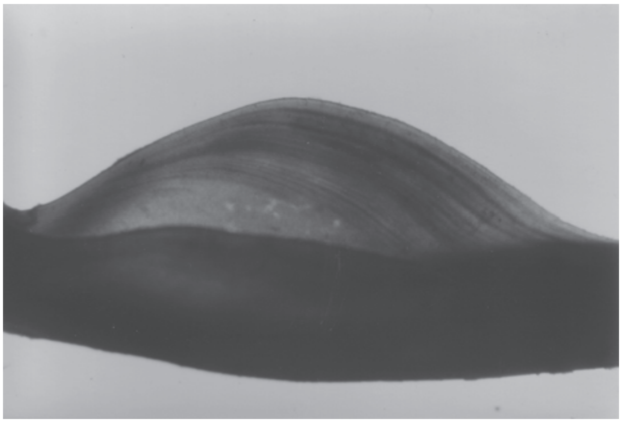

FIGURE 51 Posterior portion of the mandible of a Brown Treesnake (*Boiga irregularis*) photographed under a dissecting microscope. Lines of arrested growth (LAGs) are visible on the bone—such lines are potentially useful for aging animals by skeletochronology. In this case, however, the LAGs correspond to ecdysis cycles and thus provide little information on age in years. (Photo by E. P. Collins, U.S. Geological Survey.)

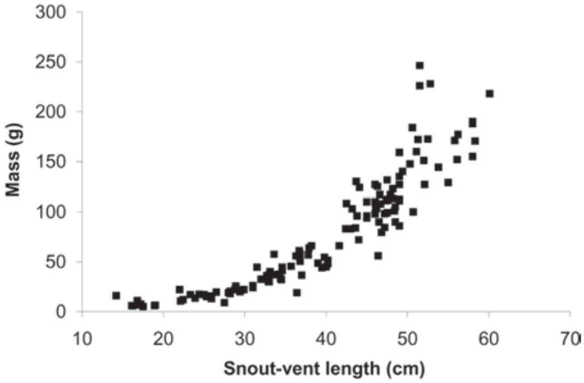

FIGURE 52 Relationship between snout-vent length and mass for the male New Mexico Ridge-Nosed Rattlesnake (*Crotalus willardi obscurus*) from a single population, illustrating absence of distinct age-size classes even in a population inhabiting a seasonal montane climate (Unpubl. data courtesy of A. T. Holycross.).

strated that the primary correlate of LAGs in the mandibles of tropical Brown Treesnakes (*Boiga irregularis*) was ecdysis, as growth slowed appreciably during each skin-shedding cycle. In contrast, a long-lived tropical lizard (*Iguana iguana*) in Panama exhibited seasonal patterns of growth in phalangeal bone, possibly corresponding to slow or arrested growth during the annual dry season (Zug and Rand 1987), and LAGs were evident in femurs from African Desert-Plated Lizards (*Angolosaurus skoogi*) from an aseasonal environment (Chinsamy et al. 1995—note that results were not validated with known-age animals).

Skeletochronology is used infrequently on squamate taxa as compared to turtles and crocodilians (see "Turtles" and "Crocodilians" in this section, below). In long-lived reptile species, the technique is complicated by two factors: (1) growth rates typically decline in older individuals, such that annuli are closely spaced and difficult to distinguish; and (2) internal bone layers may be resorbed over time. Either phenomenon could result in age being underestimated. Gibbons (1976) reviewed several older studies based on the assumption that annuli on squamate bones are deposited annually; he concluded (p. 457) that "the supporting evidence for reptiles in every instance, without exception, was based on assertion and supposition, not on solid proof, that is, not on known-age specimens. Furthermore, a disturbing amount of variation sometimes occurred in the number of rings appearing on different bones from the same specimen." More than 30 years later, this summary still serves as a warning against overconfidence in results of poorly executed skeletochronological studies.

SIZE AS AN ESTIMATE OF AGE

It is tempting to assume that one can assign ages to individuals in a population based on relative body sizes (e.g., Gibbons 1972; Fitch and Henderson 1977). This notion is based on the premise that size variation within a cohort is less than the size variation evident between cohorts. However, body size does not necessarily reflect age in reptiles (Gibbons 1976), as can be shown by regressing body size on age in known-age (from marking or skeletochronology) individuals (see Halliday and Verrell 1988). This caveat has been demonstrated for *Iguana iguana* (Zug and Rand 1987), *Thamnophis* (Waye and Gregory 1998), and *Elaphe obsoleta* (*Pantherophis obsoletus;* Blouin-Demers et al. 2002). Ridge-nosed Rattlesnakes (*Crotalus willardi*) in New Mexico have discrete annual activity periods alternating with winter hibernation periods. If body size reflects age in this population, then fairly distinct annual size classes should be evident. That is not the case (Fig. 52) for rattlesnakes measured during the same late-summer sampling period across several years or even for neonate and juvenile age classes. Instead, interindividual differences in growth rates apparently exceed the differences between age cohorts.

Rattlesnakes (*Crotalus* and *Sistrurus*) are unique in having a caudal rattle to which segments are added in discrete units as individuals shed. Shedding may occur multiple times during an individual's active season (Klauber 1956). For intensively studied populations, ages can be estimated roughly if shedding frequency is known across individuals and rattle strings are complete (Beaupre 1995). Unfortunately, terminal rattle segments frequently break off, reducing the accuracy of this method. Furthermore, in a long-term study of a population of Timber Rattlesnakes (*Crotalus horridus*), Fitch (1999) observed considerable variation in rattle segment number even within tentative age classes, including snakes that were less than 4 years old; the failure of rattle segments to predict age accurately using data from this 50-year study implies that it will rarely be a successful technique in studies of more typical temporal duration.

Turtles

Accurate age determinations are essential for calculations of growth and mortality rates, ages at maturity and recruitment, and estimates of longevity. The wide variety of approaches used to age turtles indicates that no single method is ideal for all species, ages, or size classes. Reasonable estimates of age may be obtained by using combinations of approaches, however. Turtles bear external and/or internal growth indicators, even if those indicator marks are subject to loss, erosion, or remodeling with increasing age.

EXTERNAL GROWTH MARKS

Age estimates for turtles are based on counts of growth marks or rings formed by the deposition of keratin to epidermal scute layers. A validation or confirmation that marks are formed annually is essential before rings can be termed annuli. Confirmation of growth marks as indicators of annual growth should include inter-rater agreement on the number or independent verification from animals of known age, or a known period at liberty. Counts of scute annuli appear to be reliable age estimators for freshwater and terrestrial turtles, especially those from temperate and subtropical regions, and are widely used by turtle researchers (Germano and Bury 1998). In contrast, turtles that occupy habitats with relatively stable year-round temperatures have indistinct annuli (Tinkle 1958; Moll and Legler 1971), whereas turtles in captivity or occupying oligotrophic lakes produce multiple rings each year, a reflection of their year-round food supply and feeding (Georges 1985; Kennett and Georges 1990; Tracy and Tracy 1995). Growth marks in turtles from unpredictable or unproductive habitats can also be difficult to detect and interpret, either because they overlap or because growth is suspended during periods of drought (Burbidge 1967). Growth rings are more readily seen in juveniles than in adults, in whom they can be closely spaced or even overlapping with slowed growth. Graham (1979) outlined how to interpret growth rings, and Wilson et al. (2003) critically reviewed the assumptions of growth-ring analysis.

Mark-recapture studies with individually marked turtles can be used to test for annual periodicity in growth-mark deposition by scoring the most recent growth zone with a sharpened nail to establish a benchmark. A turtle captured *n* years after scoring should bear *n* additional growth marks that correspond to periods of slowed seasonal growth, even though warmer or cooler climates may affect the rate of ectothermic growth (Germano 1994). Growth marks can be recorded physically with cast impressions of scutes taken with dental impression compound (Galbraith and Brooks 1987b), pencil shadings of the growth marks onto tracing paper (J. Parmenter, pers. comm.), or photographs (C. Guyer, pers. comm.).

Periodic growth marks may also appear in laminar keratinized structures such as claws, which can enhance age estimates for some emydids (Thomas et al. 1997); preliminary studies suggest, in contrast, that discerning such laminae in chelids is more difficult (ADT, unpubl. data). Kobayashi (2000) proposed that pigment banding on scutes in response to thermal fluctuation could be used to age *Eretmochelys imbricata,* but Tucker, Broderick, and Kampe (2001) found that pigment banding did not necessarily correspond to annual laminations.

INTERNAL GROWTH MARKS

The use of skeletochronology to estimate the ages of individual turtles (Castanet et al. 1993) has been limited because it is difficult to obtain known-aged specimens for validation. Although skeletochronology can be applied to live turtles through surgery and bone biopsy, it is more commonly used postmortem to inspect the skeletal elements (Klinger et al. 1997). Applicability of skeletochronology can vary widely, as growth marks are of considerable use for establishing age with some species (Castanet and Cheylan 1979), yet of limited use in others (Mattox 1936; Suzuki 1963; Hammer 1969; Dobie 1971). Skeletochronology is used more frequently in studies of long bones in sea turtles (Klinger and Musick 1995; Zug and Parham 1996; Parham and Zug 1997; Zug and Glor 1998). Osseous remodeling can limit the use of internal marks for aging turtles (Bjorndal et al. 1998). Ideally, biomarkers that are established by injecting a vital stain (e.g., alizarin or oxytetracyclin) into a study animal should be used for validation (Klinger and Musick 1992; Castanet et al. 1993).

CORRECTIONS FOR LOSS OF GROWTH MARKS

Erosion rates of external marks vary; for example, wear rates on plastral scutes of terrestrial species are high, as are those of species that abrade the carapace on coral reefs. Internal marks in bone are subject to remodeling as endosteal bone is resorbed. Because growth marks can be lost or resorbed, it is necessary to estimate the number of marks that may be missing. Several investigators (e.g., Sexton 1959b; Magnusson, Cardoso de Lima, Lopes da Costa, and Pimentel de Lima 1997; Parham and Zug 1997) have discussed approaches to estimating numbers of missing scute annuli for turtles. Adjustment methods used when internal bone lamina are resorbed include (1) the Fraser-Lee correction factor protocol, (2) a regression growth protocol, and/or (3) a ranking protocol (Francis 1990; Klinger and Musick 1992; DeVries and Frie 1996; Zug and Parham 1996; Parham and Zug 1997; Zug and Glor 1998). The assumptions for all correction protocols—that is, (1) that growth layers are deposited annually, (2) that the methods adequately correct for lost layers, and (3) a constant or variable allometry exists between bone growth and body size—should be validated

Studies that use complementary techniques of stable-isotope ratios (to clarify trophic level) together with skeletochronology have potential to establish relative ages or size classes when a species has a specific ontogenetic shift, the premise being that elemental signatures in bone lamina correspond to growth phases in different habitats (Snover et al. 2000).

Radiometric aging is based on the 210Pb/226Ra ratio in the calcified structure of bone. The naturally occurring radioisotope 226Ra is incorporated into the bone matrix and subsequently decays to 210Pb. The ratio of the isotopes is an index of the time elapsed since incorporation of 226Ra. Since the half-life of 210Pb (22.3 y) scales appropriately to a turtle lifespan, this isotope pair is particularly suitable for research with turtles. Informative coverage of developments in radiometric age determination can be found in the marine fisheries literature (e.g., Burton et al. 1999).

Crocodilians

For aging studies of crocodilians, individuals are marked so that they can be identified in future years. This can be done by clipping the tail scutes of hatchlings or adults according to an individual or cohort numbering system (See “Crocodilians” under “Morphological Modifications” in Chapter 9, “Marking Reptiles,” for a representative scheme). Individuals can also be identified with uniquely numbered tags affixed to the foot webbing or tail, or more securely, with a passive integrated transponder (PIT tag). PIT tags, also known as “microchips,” allow researchers to permanently mark animals internally without altering external appearance. PIT tags are injected subcutaneously using a 12-gauge hypodermic needle and syringe; they can also be externally attached using adhesives.

Implant location varies depending on the species being studied, animal size, and in some cases, the behavior of the animal. The tail is a logical implant site since one can clip crocodilians of all sizes in the same place. A commonly used implant site for *Crocodylus acutus* is the right side between the 5th and 6th rows of scutes from the rear of the vent (F. Mazzotti, pers. comm.).

Skeletochronology can be used to estimate age from growth marks in bone after validation and verification with a series of animals of known age. The approach has been applied to the long bones of sacrificed or harvested crocodilians, to osteoderms removed from free-living individuals, and also to long bones and osteoderms removed from museum specimens (de Buffrénil 1982; Hutton 1986; Games 1990; Cooper-Preston 1992; Woodward and Moore 1992; Tucker 1997). Vital staining with oxytetracycline incorporates a known marker in the bones (Hutton 1987; Roberts et al. 1988). A secondary means of validation is possible if a series of osteoderms is removed from an individual crocodile over time; the number of growth marks added between two samples is assumed to correspond to the number of intervening periods of metabolic diapause.

A major limitation of skeletochronology with crocodilians is the osseous remodeling that occurs when breeding females mobilize calcium from bone to form egg shell (Elsey and Wink 1986). The remodeling process may obliterate portions of the early growth marks in endosteal bone that are used to estimate age. Back-counting methods that attempt to estimate missing growth rings may be helpful in estimating age in some cases (Hutton 1987), but slow somatic growth after maturity may result in closely spaced growth marks that are difficult to detect.

Determining Reproductive Condition

Snakes and Lizards

Snakes and lizards display a bewildering variety of reproductive modes, seasons, and physiologies. Saint Girons (1985, p. 37) succinctly summarized this variety, stating, "[T]he modes of lepidosaurian reproduction are diverse. Thus, some species are monestrous, whereas others are polyestrous; some are seasonal, whereas others are not; some are oviparous and others are viviparous; some are bisexual and others are parthenogenetic. Also, the time of embryonic development . . . is very variable, as is the length of time between mating and ovulation." Given the range of reproductive variation and the lack of solid reproductive data for a majority of species, only limited generalizations can be made about determining sexual maturity and reproductive status of most species of snakes and lizards.

COLOR LABILITY AND REPRODUCTION

Changes in color are frequently associated with breeding in squamates, such that reproductive condition can be determined visually (reviewed in Cooper and Greenberg 1992). Most work on the relationship of color lability and reproduction in squamates has focused on diurnally active species. Physical changes in the chromatic properties of somatic surfaces can occur on short (physiological changes) or longer (morphological changes) time scales, and color changes can even be accomplished without a physical change via postural adjustment or scale movement to expose different surfaces to view (Cooper and Greenberg 1992). Male lizards commonly exhibit striking seasonal color change on the head and/or body, especially in the families Iguanidae (*sensu lato*), Agamidae, Lacertidae, Teiidae, and Cordylidae (Cooper and Greenberg 1992). Females of these families may also experience color changes during the reproductive season. Lateral spots on female *Crotaphytus collaris* and relatives, for example, turn bright orange or red during final vitellogenesis and ovulation but fade after oviposition (Ferguson 1976). While various other factors may contribute to seasonal or short-term color changes (e.g., disruptive coloration, aggressive encounters, defensive behavior, background matching, ontogeny, mimicry), the appearance of bright, visually obvious head or body colors generally occurs during times of reproductive activity in lizards and is a good indicator of sexual maturity.

SEXUAL MATURITY AND REPRODUCTIVE ACTIVITY IN MALES

Direct observations of mating and confirmed fertilization of ova are the ideal indicators of sexual maturity, but mating of most species is rarely observed in the field. Additionally, observations of copulatory behavior do not necessarily equate with reproductive maturity or successful fertilization. For male snakes and lizards, therefore, attainment of sexual maturity is usually confirmed via dissection. A time series of individuals may reveal seasonal changes in testis volume or mass relative to body size; large testis size presumably is indicative of sperm production, which occurs during the mating season (Shine 1977; but see discussion of dissociated sperm cycles, below). Testes volume can be calculated using the equation for volume of an ellipsoid ($4/3\pi ab^2$, where $a = ½$ the largest diameter and $b = ½$ the shortest diameter; James and Shine 1985). The epididymis is a highly coiled structure attached to the posterior portion of the testis, and (along with the vas deferens which proceeds posteriad from the epididymis to the cloacal region) serves as a storage organ for spermatozoa in amniotes. Upon attaining sexual maturity, males begin producing sperm, and the epididymis and vas deferens become markedly convoluted, thickened, and opaque (Fig. 53). Gross dissection of sacrificed males can thus reveal whether a specimen has reached sexual maturity. This technique is very useful for determining size at maturity for a species (e.g., Shine, Ambariyanto et al. 1999b), but it is less useful for determining reproductive seasonality (see discussion of dissociated sperm cycles, below).

Mature sperm can be collected from males (and recently mated females) with a cloacal wash. A cloacal wash is easily performed by inserting the tip of an eye dropper or plastic laboratory wash bottle spout through the vent and a few millimeters into the cloaca of the animal and then flushing the cloaca with Ringer's or comparable saline solution. A few drops of cloacal fluid or wash liquid are smeared onto a microscope slide. Microscopic examination can be fairly simple (e.g., reflected light under a 60–400X dissecting scope; Fitch 1960; Aldridge and Brown 1995) or can involve more complex histological techniques (see "Turtles" and "Crocodilians," below). Hoser (2008) espoused "masturbating" snakes by stroking the caudal region of presumptively mature males, which can result in ejaculation of semen. Although Hoser (2008) envisioned this as a technique for artificial insemination of female snakes, we suggest that the collected semen could alternatively be

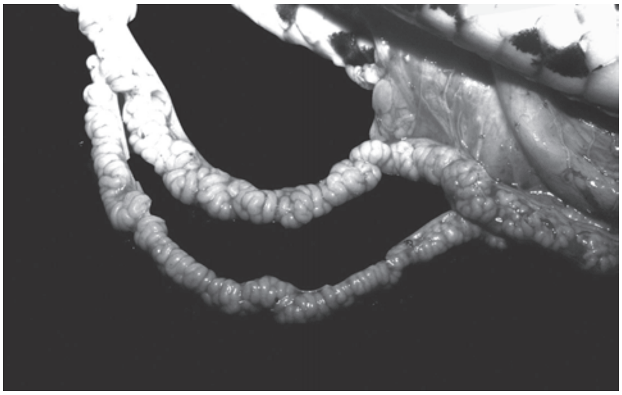

FIGURE 53 Thickened, convoluted vas deferens of a male Yellow Anaconda (*Eunectes murinus*) from northeast Argentina, dissected away from the body for clarity. Thickened vasa deferentia are indicators of sexual maturity in many male reptiles. (Photo courtesy of T. Waller.)

transferred to a slide for microscopic confirmation of mature spermatozoa.

The stage of spermatogenesis in a male can be determined from histological examination of testes and associated tissues taken from sacrificed males (for methods of sample preparation, see Wilhoft 1963; Aldridge 1979; Zug et al.1982; Aldridge and Brown 1995; and Ramírez-Pinilla et al. 2002). In subtropical and tropical reptiles, spermatogenesis may be continuous throughout the year, so presence of mature sperm is not necessarily an indicator of the mating season even when the mating season is seasonal (Zug et al. 1982). Alternatively, in many reptiles the spermatogenic cycle, during which sperm is produced, is dissociated, or temporally separated, from the mating season. This is most common in temperate zones, where males may overwinter with immature sperm in the testes or mature sperm stored in the vas deferens (Saint Girons 1985). Those sperm are used for mating in the next active season. The presence of mature sperm may thus be a reliable indicator of sexual maturity in males, but it is less reliable as an indicator of mating season.

Male squamates, and possibly *Sphenodon* (Gabe and Saint Girons 1964), are characterized by having a *sexual segment in the kidney* (SSK). The SSK is sensitive to androgens (e.g., testosterone) and secretes seminal fluid that is transferred to the female during copulation (Regaud and Policard 1903; Bishop 1959; Fox 1977; Sever et al. 2002). The SSK hypertrophies during the mating season in many species (Saint Girons 1982). Because spermiogenesis is often temporally dissociated from the mating season in snakes (Saint Girons 1982), the SSK may be a more reliable indicator of the mating season in that group than is the presence of mature sperm. The location of the SSK relative to the kidney and ureters varies among squamate taxa, and the function of fluid secreted from the SSK is debated (Fox 1977). Thus far, the SSK has only been examined in dead animals, although laparoscopy could conceivably be used for visual determination of hypertrophy in live individuals. At a gross level, the diameter of the SSK can be used to examine seasonal hypertrophy (Aldridge and Brown 1995). However, diameters must be adjusted for the body size of the individual via analysis of covariance or residual analysis. Activity in the SSK can also be determined histologically. A testis and the attached anterior portion of the kidney are removed, fixed, and prepared appropriately for examination under a standard (Aldridge 2001) or electron (Sever et al. 2002) microscope.

REPRODUCTIVE MATURITY AND ACTIVITY IN FEMALES

As noted for males, direct observations of mating of snakes and lizards are uncommon, as are observations of oviposition or parturition. The presence of developing offspring, however, is often easily detected. Shelled eggs are visible through the ventral skin of many geckos, anoles, and other thin-skinned lizards, although care must be taken to distinguish them from ventral fat bodies. Gravid females of diverse taxa may appear heavy bodied, with noticeable distention in the posterior portions of the body. In contrast, distention caused by large ingested prey items generally occurs at midbody (this phenomenon is most common in snakes, which tend to eat relatively large prey items). For elongate squamates, such as snakes and pygopodids, gentle palpation of the abdomen can reveal the number and size of follicles or embryos. The female is held belly up and a thumb run along the venter, allowing the eggs to slide under the thumb one by one (Fitch 1987). Determining the number of offspring in this manner is usually most effective with oviparous species because the embryos in viviparous species may not be arranged in a strictly linear fashion. Fitch (1965) reported, however, that female snakes that were frequently palpated produced smaller litters, so this method must be used judiciously. Males of some species deposit copulatory plugs in the cloacas of females during the latter stages of copulation; these plugs prevent subsequent males from achieving successful intromission (Devine 1975, 1977). Copulatory plugs may be visible when the female is in hand, appearing as a beige or yellowish semi-mucilaginous mass obstructing the female cloaca. A plug indicates that the female has recently mated but provides no sure evidence of fertilization.

Additional indicators of reproductive status can be observed in dissected females. The oviducts of females that have previously reproduced are thick and muscular and appear “stretched,” compared to those of virgin females, indicating that the females are sexually mature even if maturing ova or embryos are absent (Fig. 54). Counting and measuring the diameter of developing ova or ovarian follicles can provide information on timing of ovum development. Follicles can be grouped by size class, with females considered reproductively active if follicles exceed a given diameter (Zug et al. 1979; Butler 1993). Once ova mature, the follicles rupture and ova migrate to the oviduct. They leave behind the remnants of the follicles, which develop into hormone-secreting *corpora lutea* (also known as yellow bodies); the number of corpora lutea indicates the number of ova released in the previous reproductive bout. Corpora lutea eventually degenerate and become difficult to detect after oviposition/parturition is complete; in some taxa (e.g., *Boiga irregularis*) they degenerate within days (G. H. Rodda, pers. comm.). In oviparous taxa, deposition of the eggshell often occurs within hours or a few days prior to oviposition (Palmer et al. 1993), such that the presence of shelled eggs in the oviduct indicates imminent oviposition.

Laparoscopy on live animals is a feasible alternative to dissection of sacrificed females for determination of reproductive condition in some snake and lizard species. Cree et al. (1991) were able to distinguish between atretic, early, mid-, and late vitellogenic stages of egg development, as well as preovulatory, ovulatory, and gravid states in tuataras using laparoscopy. This procedure should be applicable to most of the larger species of snakes and lizards as well. Ultrasonography provides the same information as laparoscopy, but it is noninvasive and allows for repeated examinations of the same individual through the reproductive cycle (Chiaraviglio et al. 1998; DeNardo and

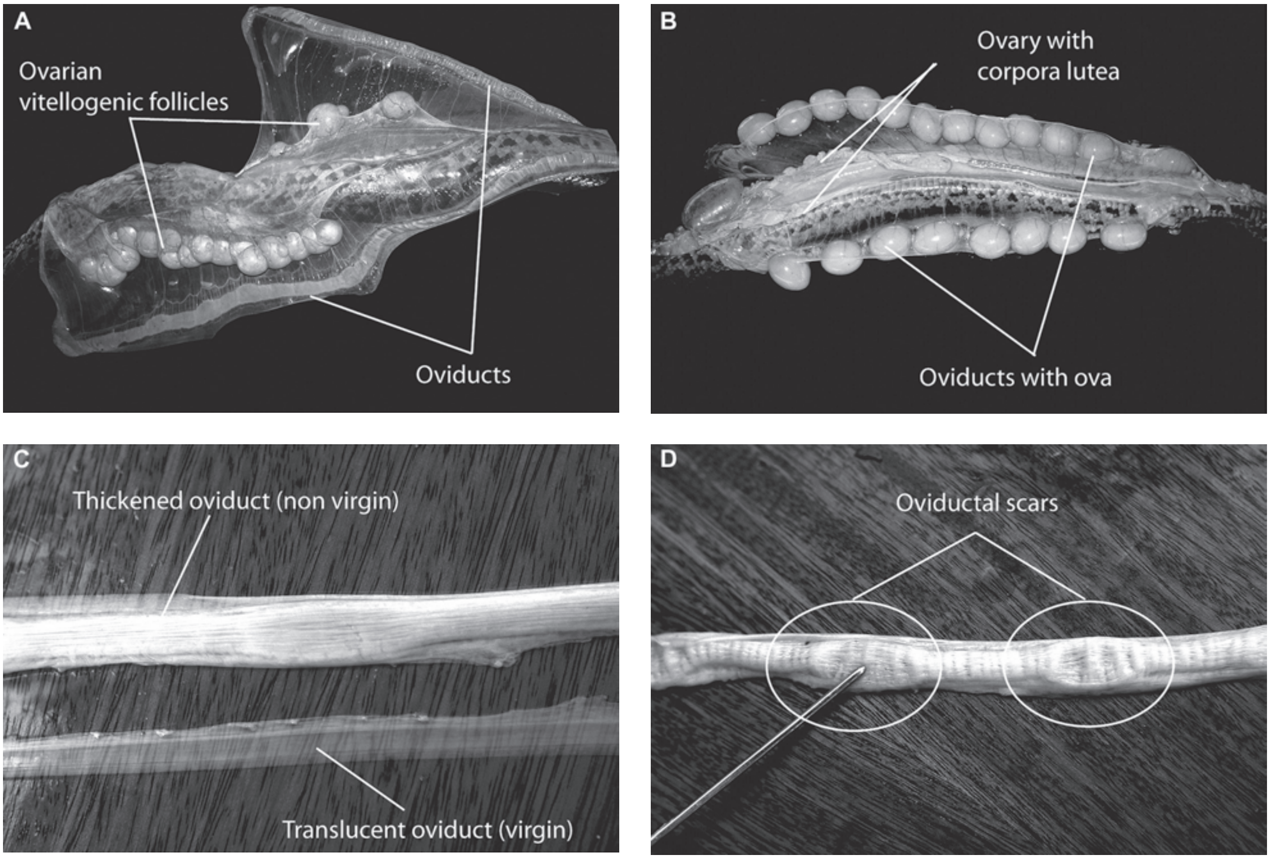
A
Ovarian vitellogenic follicles
Oviducts
B
Ovary with corpora lutea
Oviducts with ova
C
Thickened oviduct (non virgin)
Translucent oviduct (virgin)
D
Oviductal scars

FIGURE 54 Reproductive tracts of female Yellow Anacondas (Eunectes murinus) from northeast Argentina, dissected away from the body for clarity: (A) Nonvirgin prereproductive female with ovarian 2° vitellogenic follicles and oviducts showing scars from previous ovulatory events. (B) Female that has recently ovulated. (C) Comparison of oviducts from virgin and nonvirgin females. (D). Oviduct from a nonvirgin female showing scars from previous ovulations. (Photo from Waller et al. 2007; reprinted with permission.)

Autumn 2001; Bertona and Chirarviglio 2003). Until recently, ultrasonographic equipment was not easily portable in the field but could be used if a portable generator, large battery, or automobile with available electric power was available (D. DeNardo, pers. comm.; see "Turtles" and "Crocodilians," in this section and below, for additional information). Ultrasound imaging systems are now available in field-portable units the size of laptop computers; Gilman and Wolf (2007) evaluated one such system for estimating reproductive effort in small lizards. They concluded that the accuracy of egg counts decreased somewhat with increasing clutch size and that estimates of egg and clutch volumes were only moderately accurate. Errors decreased in magnitude with increasing operator experience, and a significant proportion of remaining errors were due to species-specific challenges.

DETERMINING REPRODUCTIVE MATURITY AND ACTIVITY FROM BLOOD SAMPLES

Blood samples can often provide information on reproductive status through seasonal changes in blood plasma components (e.g., hormones or other substances associated with reproduction). Such changes, however, have been thoroughly investigated in only a few reptile taxa. Among female snakes and lizards, (1) increased levels of gonadotropin generally signal the beginning of an ovarian cycle and lead to yolking of ova; (2) vitellogenesis may also be associated with increased levels of estradiol 17-β, testosterone, total calcium, vitellogenin, and total circulating protein; and (3) elevated progesterone may signal ovulation and/or gravidity. Among males, testosterone tends to be elevated during sperm production, but this may not be indicative of the breeding season. Sex steroid metabolites from fecal and urinary deposits have been analyzed as an indicator of reproductive activity in mammals (e.g., Monfort et al. 1993). To our knowledge, this methodology has not been attempted with reptiles, largely because most reptiles urinate and defecate infrequently (D. DeNardo, pers. comm.). Detailed discussions of hormonal controls of reproductive behavior in reptiles can be found in Licht (1984), Moore (1987), Moore and Lindzey (1992), and Whittier and Tokarz (1992).

Turtles

In turtles, sexual maturity can be determined from the appearance of external sexual characteristics, by direct or indirect inspection of the gonads, or by monitoring gamete production and storage. For female turtles, maturity is defined as the ability to produce eggs in the forthcoming season. For males, it is defined as the production of spermatozoa. Neither size nor age is an absolute indicator of maturity, because both minimum age and size at maturity vary geographically and within a population. Many researchers distinguish between functional and physiological maturity, because functional reproductive organs are not necessarily in use yet. Female chelonians, for example, may undergo cycles of vitellogenesis during puberty yet resorb the follicles before ovulation of a primiparous clutch.

Palpation can be used to detect eggs and assess sexual maturity in females. With the turtle held upright, fingers are inserted into the inguinal space anterior to the hind limb, and the female is gently jiggled; oviductal (Smith et al. 1989) eggs (if present) produce a tapping sensation against the fingertips.

Sexual characteristics are expressed in response to circulating gonadal hormones. Levels of circulating testosterone can indicate whether a male is mature (or not) as well as his reproductive status. With females, levels of circulating estradiol, FSH, LH, or calcium can signal the physiological stage of reproduction. The phenology of the reproductive cycle is beyond the scope of this chapter, but useful summaries can be found in Owens (1995, 1999). Reproductive cycles in turtle species can vary from perennial, with multiple clutches produced each year, to complex, with clutches produced at multiyear intervals controlled by levels of stored lipids, which are likely influenced by environmental conditions.

Male maturity is generally inferred once the tail elongates at puberty, but a visual inspection or biopsy of the gonad is required for confirmation. Laparoscopy is preferred over sacrifice of an animal. Semen has been collected by electrically stimulated ejaculation in a representative cross section of sea turtles, tortoises, and freshwater turtles, allowing microscopic analysis for the presence of mature sperm (Platz et al. 1980).

Nondestructive methods for studying sex or reproductive status in live turtles (and other reptiles) are listed in Table 13. Apart from studies by Wyneken (2001), MRI and CAT scans have not been used routinely in field studies of turtles, because they are expensive and the equipment lacks portability. The methods in use for field studies include X-radiography, ultrasonography, laparoscopy, and analysis of blood or tissue samples.

X-radiography has long been used to count shelled oviductal eggs in gravid females and to measure their linear dimensions (Burbidge 1967; Gibbons and Greene 1979). Kuchling (1998) expressed concerns about long-term health consequences of exposing females and developing embryos to ionizing radiation. Presumably, the threat to chelonians is negligible if cassette films with rare earth screens, which require less radiation during exposure, are used instead of Ready Pack film (Hinton et al. 1997). The recommended distances and length and intensity of X-ray exposures are species specific, but guidelines are available for small- to medium-size animals (Silverman and Janssen 1996; Hinton et al. 1997).

An alternative method, ultrasonography, was successfully introduced for imaging *Pseudemydura umbrina* (Kuchling 1989), and the approach was quickly adopted for use with other turtles. Ultrasound analysis is generally conducted with a small linear or convex transducer probe that fits (field depths as appropriate to the turtle's internal volume) into the inguinal pocket. To minimize sonic echoes from the carapace, turtles are submersed in a water bath, scanned through a gel-filled glove, or simply scanned with a surface film of coupling gel, depending upon the turtle's size. Ultrasound is used primarily to monitor vitellogenesis and egg production but can sometimes reveal sex when the gonads are distinct and resolved by the imaging thresholds for detection (practical detection limits for soft organs are often less than stated specifications). New versions of color Doppler ultrasound are available, but these are used mostly by veterinarians rather than field biologists.

Laparoscopy is the surgical insertion of an optical scope through the body wall into the body cavity for direct viewing of the gonads *in situ* (Wood et al. 1983; Limpus and Reed 1985). With appropriate equipment, individuals as small as 50-mm straight-line carapace length can be sexed by laparoscopy (Rostal et al. 1994). Although laparoscopy is minimally invasive, it is time consuming and requires specialized equipment, and the practitioner must have proper training and a thorough knowledge of turtle internal anatomy. As with all surgical procedures, the risk of mortality increases compared to blood or tissue biopsies. Although laparoscopy is not widely used, it is the standard approach for evaluating the accuracy of other methods of sex determination. A good overview of laparoscopy (and a variant known as soft-tissue laparotomy) is found in Kuchling (1999).

Crocodilians

Reproductive maturity in female crocodilians can be determined via laparoscopy, ultrasound, and cloacal examinations, and in males, with penile swabs. In this section we focus on studies of *Crocodylus johnstoni* (Webb et al. 1983; Limpus 1984; Tucker and Limpus 1997), which has been a subject for all the methods we mention. In fact, all the methods are applicable to most crocodilians, whose general anatomical appearance is similar (Richardson et al. 2002). Field laparoscopies are performed with the crocodilian restrained in dorsal recumbency to a board or ladder. The restraint board should be tilted head down to allow gravity to position the viscera for ease of viewing. Some researchers prefer to work with a local anesthetic for smaller individuals (<2 m total length). Larger individuals can be sedated (see Lloyd 1999); at least 24 hours are required for recovery after surgery. Details of field laparoscopy, including equipment and procedures, are outlined in Limpus (1984).

Cloacal examinations consist of inserting a finger via the cloaca into the lower intestine, voiding any fluids, and palpating through the intestinal wall for follicles or eggs. Females are considered to be sexually mature if soft- or hard-shelled eggs can be felt by palpation through the cloaca, if the cloaca is flaccid as a result of recent egg passage, if the oviduct is enlarged and highly convoluted, or if vitellogenic ova or corpora lutea are observed via laparoscopy. A convoluted oviduct but no enlargement of previtellogenic follicles identifies a pubescent female. A white straight oviduct identifies a juvenile female.

Male maturity is determined by visual examination of the testis and associated ducts by laparoscopy. Adult males have a pendulous sausage-shaped testis that protrudes from the body wall and a convoluted epididymis enlarged with sperm. Pubescent males have an elongate and ellipsoidal testis but little evidence of a convoluted epididymis. Juvenile males have an elongate but flat testis and a small straight vas deferens. Breeding activity is evaluated by microscopic examination of sperm smears. A small spatula is gently wiped along the penile groove to collect mucous-like fluid. The fluid is smeared across a numbered microscope slide with the edge of a second microscope slide. Slides are placed into a covered slide box with a few drops of buffered formalin providing vapor for fixation (too much liquid may wash the sample off the slide). Slides are processed in the laboratory using standard histological methods (hematoxylin and eosin staining). The slides are examined under a microscope and coded from S0-S3 for visual estimates of none, few, common, or abundant sperm, respectively. Adult males are judged as breeding if the sperm smear shows active spermiogenesis (S2 or S3).

A portable generator or a 12V battery with a power inverter can be used to power veterinary ultrasound units. Units with a 5.0- or 7.5-MHz linear transducer will scan body depths from 10.0 to 12.5 cm; a 3.5-MHz probe provides a deeper scanning range for larger crocodilians. Females are restrained on their backs, and a finger is inserted into the cloaca as external pressure is applied to the lower abdomen to void urine and feces. Ultrasound coupling gel is applied to the abdomen, and the transducer is scanned along the ventro-lateral portion of the abdomen to look for echogenic follicles or eggs. Left and right sides are scanned independently to simplify counting and to minimize acoustic shadows from gastralia along the belly or osteoderms along the flanks. Images can be frozen on screen and measured with built-in electronic calipers. Ultrasound scans should be conducted before laparoscopy to avoid possible sonic interference from air remaining in the abdomen after laparoscopy.

Ultrasound diagnosis accurately assesses reproductive state if the crocodiles are examined prior to oviposition and after the intestine is voided. A broad window exists for detecting breeding status via ultrasound because vitellogenesis extends for several months in crocodilians (Lance 1989; Vac et al. 1992), and the period from ovulation to oviposition lasts an additional 3 to 4 weeks. It is possible to count eggs or follicles in gravid females of many smaller or less fecund species. Ultrasound does not provide an accurate estimate of clutch size in species with large clutches, because the egg masses are either difficult to image or lie within an acoustic shadow. Vitellogenic follicles can inflate estimates of reproductive output if follicle counts, rather than egg counts, are used, because of the potential for later follicular atresia. In general, images of previtellogenic follicles, corpora lutea, and late-stage atretic follicles can seldom be distinguished, possibly because of detection thresholds.

Although portable X-ray machines would be feasible in field studies of crocodilians, most researchers simply count, measure, or collect eggs after oviposition.

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PART THREE

SAMPLING REPTILE DIVERSITY

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CHAPTER ELEVEN

Techniques for Reptiles in Difficult-to-Sample Habitats

ROBERT E. LOVICH
Chapter Coordinator

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Overview

Robert E. Lovich

This book deals with sampling and other aspects of field research on reptiles. Not surprisingly, the disparities in size and methods of locomotion between reptiles and humans constrain the ability of the latter to sample the former and often interfere with the regular, methodical collection of information (capture or observation) that scientific protocols and methodologies require. The partial or complete inability of humans to negotiate certain types of habitat (e.g., deep water, unstable rock piles, treetops) and/or the ability of reptiles simply to avoid detection and capture in some habitats put humans at a disadvantage compared to reptiles that are well adapted to their particular habitats. In this chapter, we deal with sampling methods and procedures for a subset of habitat types in which we believe reptiles to be especially difficult to sample.

While we do not provide comprehensive coverage of all the difficult-to-sample habitats or reptiles, we present wide taxonomic and geographic coverage, along with a host of references useful for a project designer. We also provide names of individuals from whom additional information on sampling strategies may be sought. Many of the authors represented in this chapter are working to develop novel sampling strategies or to improve existing ones. Without doubt, readers of this chapter will also develop new strategies. Whether reptiles live underwater, underground, high in a forest canopy, sheltered within a mountain of rock, or in some other seemingly inaccessible place, they can be repeatedly and successfully collected and/or detected with methods currently available.

Rock-Dwelling Reptiles

Robert E. Lovich and Aaron M. Bauer

Sampling reptiles in rocky habitats is challenging. The dense and impenetrable nature of piles of rocks and boulders makes it difficult to locate and extract reptiles living within and among them. When searching for or monitoring *saxicolous* reptiles (from the Latin *saxum,* meaning rock, and *–cola,* meaning inhabitant; syn. *rupicolous* from the Latin *rupes,* also meaning rock), it is important to determine how the target species use the habitat and how their natural histories relate to it. Rocky habitats can provide reptiles with protection from predators, refuge from the physical environment, foraging sites (including for preying on other saxicolous animals), and breeding and denning sites; they can also contribute to reptile thermoregulation (Cowles and Bogert 1944; Huey et al. 1989; Webb and Shine 1997, 1998a, 1998b, 2000; Kearney 2002; Shah et al. 2003, 2004; Quirt 2006). Many rock-dwelling species prefer or are completely restricted to a particular type (or types) of rock (e.g., granite, sandstone, limestone, etc.) or rock crevice, because of its size, exposure, thermal qualities, or other features (Schlesinger and Shine 1994a, 1994b; Bauer and Sadlier 2000; Lovich 2001). Indeed, expansive rocky habitats that appear uniform to an untrained human observer generally provide a diversity of discrete types of microhabitat for saxicolous reptiles. The long-term stability of the rock habitat and its evolution are also important considerations. Some

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species require geologically old and stable exfoliating rocks such as those along high ridges or on tops of hills, in contrast to the more dynamic habitats in valleys and canyons that may be seasonally flooded or subject to landslides or tectonic activity in some regions (Lovich 2001).

Prior to sampling an area for saxicolous species, the investigator should thoroughly understand the thermal requirements of the target species and the temperature range and seasons within which it is active. Successful location or monitoring of species can be maximized by searching during their specific daily and seasonal activity periods. Saxicolous habitats can be sampled in a variety of ways. The methods described below are by no means comprehensive, but they should provide an overview of techniques to be considered when developing the sampling plan for a project. Additional information about field techniques relevant to saxicolous reptiles can be found in Simmons (1987, 2002) and Bennett (1999), and in Chapter 5, "Finding and Capturing Reptiles," and Chapter 13, "Standard Techniques for Inventory and Monitoring," in this volume. The specific sampling method or methods to be used should be chosen carefully to maximize sampling efficiency.

Rock Flipping

Manual rock flipping is a popular and widely used method for sampling reptiles in saxicolous habitats. This method involves moving or lifting rocks—including rock sheets or flakes—to expose reptiles that may be hidden beneath or within them. Rock flipping is most useful when collecting specimens, but the technique can be used to gather baseline data or to compile a species list for a particular area. It is an effective way for a surveyor to search through rocks at the surface or subsurface level, and it has been utilized extensively by herpetologists. When employing this method, investigators should consider and record the microhabitat used by the target species, because many reptiles prefer particular types of rock-crevice habitat (Figs. 55 and 56). Whether the rocks lie on dirt or on other rocks, whether they are vertical or horizontal, their slopes, aspects, and thermal characteristics, as well as the amount of space beneath them, are all important considerations that can help to maximize search efficiency and minimize search effort and habitat disturbance when looking for specific species. Field investigators should also be aware that repeated sampling or collecting in the same rocky habitats has been shown to result in fewer reptiles and decreased relative abundance, and to have impacts on reptile species, sex, age-class, and seasonal use (Goode et al. 2004, 2005).

One constraint on rock flipping is the size of rock that the surveyor can lift. Large rocks or boulders, as well as smaller rocks deeply buried in their surrounding substrates, may be immovable. Rock flipping is generally not useful for long-term monitoring studies, because maintaining the integrity of the habitat and a high likelihood of recapturing the same individual reptiles after the habitat is repeatedly disturbed can be difficult (Schlesinger and Shine 1994a, 1994b; Goode et al. 2004, 2005).

Tools such as crowbars and pry bars are sometimes used to facilitate manual movement of rocks that would otherwise be unmovable. Use of such tools disturbs and can destroy features of rocky habitats, and we do not recommend employing such methods except under special circumstances (e.g., salvage sampling). In many regions of the world, governing agencies expressly forbid the use of equipment to break or move rock piles for collecting purposes. An investigator should thoroughly review local laws and regulations before using any tools to facilitate sampling. The surveyor should be mindful that rock flipping, in addition to needlessly degrading or destroying habitats when recklessly employed, is potentially detrimental to resident species in other ways. Accidentally crushing animals when lifting or moving rocks is always a possibility; however, crushing what lies beneath a rock can be minimized by lifting the rock slowly and cautiously and looking for reptiles underneath, as you lift. After a rock is lifted a few centimeters, it can be wedged in position with a small stone and the area underneath it searched with the aid of a flashlight or mirror. Many rocky habitats are the result of thousands of years of weathering, and moving rocks that are tightly pressed together can break the moisture seal created by mosses, lichens, and detritus that have accumulated along the rock margins. A moisture seal can be critical for animals trying to avoid desiccation, and it can insulate them from extreme outside temperatures. It can also prevent rainwater from periodically flooding a rocky refugium. Once a rock is removed from its original location, the microhabitat that was present beneath it will be altered significantly. Habitat disruption can be minimized if rocks that are moved are returned to their original positions. We exhort anyone using manual rock flipping methods to "always put the rocks back!"

Visual Surveys

Investigators carry out visual surveys for saxicolous species to document the presence or absence of species as part of transect surveys, visual encounter surveys, or other monitoring protocols (see Chapter 13, "Standard Techniques for Inventory and Monitoring"). Simply walking through an area and noting the location of reptiles during the day or at night can provide data on species' distributions and their presences or absences at particular sites or in particular microhabitats. Such data can be used to develop a reptile species list for an area. Reptile sign, such as feces or shed skin, can also be used to identify the presence of certain species. Droppings at or near the apex of exposed rocks with a view often signal the presence of males of territorial diurnal lizards. In contrast, large accumulations of feces or nitrogenous waste near crevice openings or overhangs suggest the presence of nocturnal forms, such as geckos. Rupicolous snakes often use crevices or rock edges to assist in the removal of skin in ecdysis. Shed skins found in crevices or between rocks can provide species-level identification if they are reasonably intact.

Lantern walking at night is a widely used and effective method for monitoring nocturnal and/or crepuscular reptiles in saxicolous habitats. This method involves illuminating habitats at night while searching for resident reptiles. Lantern walking is especially useful for collecting or simply for documenting the presence of nocturnal species at a site. Although this technique is most useful for monitoring nocturnal species, it can also be used to detect sleeping or inactive individuals of diurnal species. Lantern walking can be used as a type of visual transect survey or simply to augment diurnal visual surveys or other sampling methods. Many artificial-light sources are available commercially, including battery-operated headlamps, gas lanterns, flashlights, and rechargeable lights

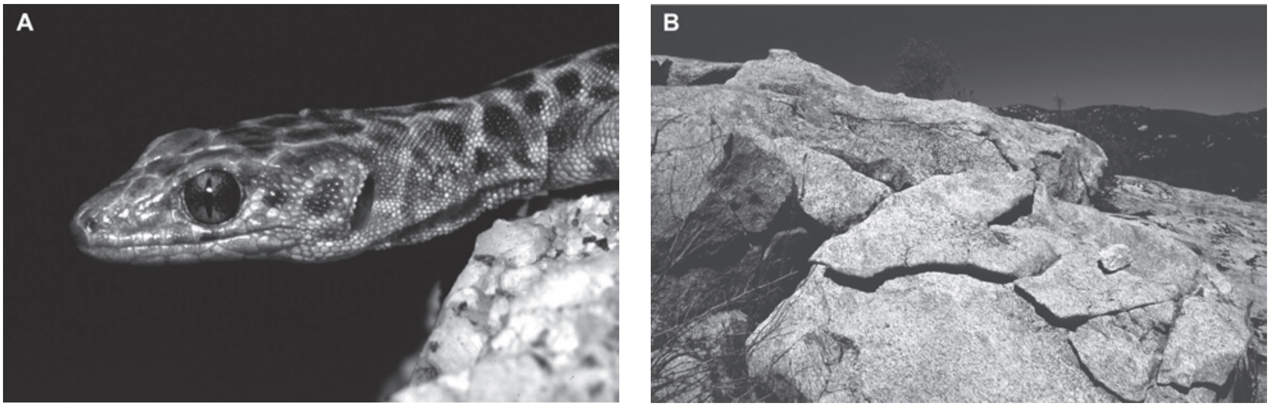


FIGURE 55 (A) Head of an adult Granite Night Lizard (*Xantusia henshawi*). Note the dorsoventrally compressed body typical of crevice-dwelling reptiles. (B) Rocky outcrop in San Diego County, California, which is typical habitat for Granite Night Lizard, Granite Spiny Lizard (*Sceloporus orcutti*), Rosy Boa (*Lichanura trivirgata*), Speckled Rattlesnake (*Crotalus mitchellii*), and other reptile species. (Photos by R. Lovich.)

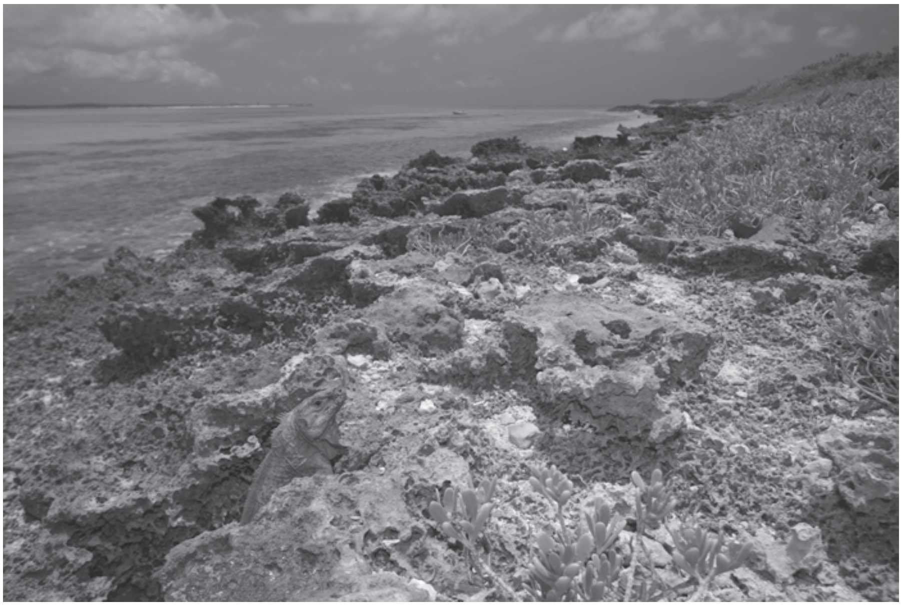


FIGURE 56 Northern Bahamian Rock Iguana (*Cyclura cychlura*) emerging from a crevice in its rocky outcrop habitat on San Salvador Island in the Bahamas. (Photo courtesy of R. Toft ©, reprinted with permission.)

of various types. The artificial-light source selected should provide adequate illumination to detect the target species and yet be of a size suitable for the surveyor to carry during the rigors of fieldwork.

Alternatively, an investigator can use a night-vision device that operates either by magnifying ambient light or supplementing it with infrared light. Night-vision devices are available in monocular or binocular form and can be worn as goggles with a head mount. Newer-generation devices may allow investigators to identify species; older (and more affordable) models lack the resolution to permit such identification, although they can be used effectively to scan rock faces or crevice openings for reptile activity.

Noosing is a method in which a simple loop of string, dental floss, or light-test fishing line fastened to the end of a rod or pole is used to capture reptiles (but see “Noosing,” in Chapter 5). This method is most useful for relatively small tetrapod reptiles. Snakes, turtles, and other reptiles are too narrow, too wide, too heavy, or otherwise not well suited for capture by this method. Many reptiles, although wary of humans, will allow an observer to approach to within some limited distance. By understanding and respecting the approach distance for a target species and employing a little stealth, an observer can usually get close enough to noose the animal. A pole of the required length with a noose at the end can be lowered and drawn around the neck of the reptile. At the moment the noose is lowered over the reptile’s head, the rod is jerked upward, and the noose tightens around the neck of the reptile, allowing it to be captured in a safe and effective fashion. Noosing rarely causes injury, although occasionally an animal will choke or be hurt while dangling from the noose. To prevent asphyxiation or other harm, reptiles should be removed from the noose as soon as possible and then processed. Soft-skinned species or those capable of gripping tightly to the substrate (e.g., geckos) are best noosed and then lifted from the substrate by hand.

In enclosed rocky spaces or rock crevices, a modified noose is most effective. A stout fishing line is doubled over and passed through a short (approx. 30–40 cm), stiff copper tube. The distal end of the tube is crimped so as to allow the bend of the fishing line to act as a noose when pulled from the proximal end. The noose must be stiff enough to retain its rigidity but ductile enough to permit bending to accommodate the architecture of a particular crevice. With the noose pulled taught, the copper tube serves as an effective crevice probe. The use of hooks to remove larger lizards from crevices is documented in Bedford et al. (1995).

Firearms and Projectiles

Another method, used primarily for collecting reptiles, is to shoot a target organism. The type of gun used should be chosen to maximize successful capture of the target species while minimizing the amount damage to the specimen. This method is controversial and potentially dangerous, or even lethal, both to the target species and the surveyor. The use of any type of gun for monitoring or field collection should be well justified and carefully planned in advance, with safety as the highest priority for all parties involved.

Air-powered and higher caliber guns are extremely effective for the capture of reptiles, but they are dangerous to the reptiles, surveyor, and other persons and animals in the area. Because guns can cause damage to the specimen, the surveyor should choose a caliber appropriate for the capturing the specific target reptile while minimizing the amount of damage to it. We highly recommended that anyone using firearms take a gun safety course and review carefully and adhere to local gun laws and regulations. Some countries expressly forbid the use or possession of any type of gun, and nearly all countries strongly regulate possession and/or use of firearms.

For millennia, indigenous peoples around the world have used blowguns and bows and arrows to capture reptiles. An investigator working in an area inhabited by indigenous people should consult them about methods for finding and capturing reptiles (see also "Collaboration with Local People for Sampling Reptiles," in Chapter 5). For blowguns on the commercial market, one can purchase both lethal (pointed-tips) and nonlethal (blunt-tipped) projectiles. Both types are useful for capturing reptiles in a variety of habitats (see "Arboreal Reptiles," below), including saxicolous habitats. The blowgun and projectile to be used should be selected after careful consideration of their functions and the objectives of the study.

Researchers can effectively collect small reptiles (≤20 g) by shooting them with rubber bands. Large, broad bands (size 107) are particularly effective when shot off of the thumb or forefinger and, depending on the skill of the collector, can be used to stun or kill lizards at distances of up to about 6 m. Rubber band guns (single- or multiple-band models) are also available commercially. Such guns tend to be quite accurate at ranges even greater than 6 m, but because they use much thinner rubber bands, they are not as effective as collecting tools. Rubber bands are cheap, quiet, safe, and reusable. In order to relocate used bands, it is advisable to mark them with bright colors that will stand out against a typical rocky background. Because "rubber-banding" is associated with high mortality rates (Vargas et al. 2000), the technique is not appropriate for long-term studies or recapture surveys.

Trapping

Pitfall trapping is a method widely used to sample terrestrial vertebrates (see "Funnel Traps, Pitfall Traps, and Drift Fences," in Chapter 5, and "Pitfall-Trap Surveys," in Chapter 13) and has been employed in many areas around the world. However, the efficacy of this sampling method for capturing saxicolous reptiles is questionable. For one thing, the habitats of many saxicolous species are so specialized that an animal seldom ventures far from its home area. Second, pitfall traps are usually placed in areas where holes can be dug deep enough for the traps to be set, which generally excludes rocky areas. Thus, saxicolous species cannot be captured reliably using this method. Given the large amounts of time, person-hours, and costs of such sampling, we do not recommend it for sampling exclusively saxicolous reptiles.

The use of "sticky traps" (also known as "glue traps") designed and marketed for the capture of pest insects and rodents is an alternative trapping method that can be useful for the capture of some saxicolous reptiles (Bauer and Sadlier 1992; Rodda et al. 1993). Sticky traps can be placed near the entrance to crevices or in other potentially "high traffic" areas. Traps should be checked at least once a day, as entrapped reptiles are susceptible to heat stress, dehydration, and predation by ants, arthropods, and other vertebrates (which themselves may become trapped; Glor et al. 2000). Animals can be removed from traps by gently coating them with vegetable oil and working them free of the glue. Although mortality rates from glue trapping are reportedly high (Glor et al. 2000; Vargas et al. 2000), prompt and careful checking and cleaning of the traps minimize injury and death. Because the efficacy of glue traps decreases with the accumulation of sand, soil, and/or moisture on the adhesive surface, these devices are inappropriate for long-term trapping programs unless they can be replaced at frequent intervals.

Traps can be baited in some circumstances, especially for species that respond to fruit, carrion, live arthropods, or live vertebrate prey. Baiting has been used successfully in conjunction with glue trapping for saxicolous lizards of the genus *Platysaurus* (flat lizards; Whiting 1998) as well as for *Eulamprus quoyii* (Eastern Water Skink) from rock crevices along creeks (Downes and Borges 1998). Under such circumstances, traps placed close to a crevice or retreat entrance may be the most successful. Other techniques reported to be effective for some rupicolous forms are adhesive devices or grips at the ends of poles, sticks, or tubes slender enough to enter narrow crevices (Durtsche 1996).

Coverboards (see "Sampling with Artificial Cover," in Chapter 13), have been shown to be effective as a method of estimating reptile and amphibian biodiversity (Grant et al. 1992) and of capturing a variety of species (Hoyer and Stewart 2000). Potentially, the boards could be used to attract saxicolous species if placed against, upon, or adjacent to rocky habitats. However, the method has not been widely used to sample rocky habitats, so little is known about its efficacy with respect to saxicolous species. Nevertheless, coverboards are inexpensive, require little maintenance, and may be useful when deployed in conjunction with other sampling methods. The size, thickness, and substance of the coverboard must be carefully selected to ensure an appropriate thermal regime (e.g., metallic and/or very thin materials should be avoided) and permanence (e.g., boards made of light, low-density, or high-profile materials may blow away in

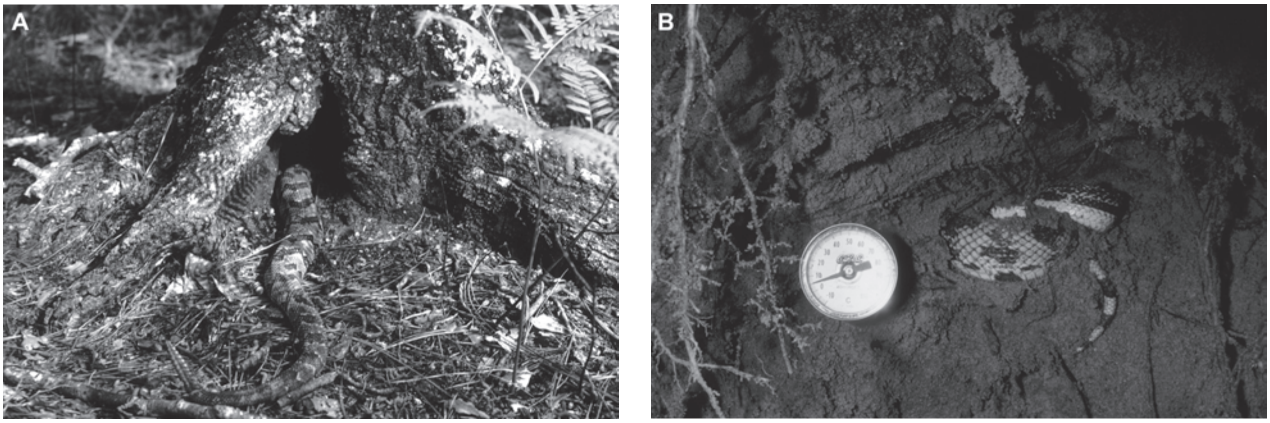


FIGURE 57 (A) Timber Rattlesnake (*Crotalus horridus*) entering a den, Pine Barrens, New Jersey. (B) Pinesnake (*Pituophis melanoleucus*) in hibernation within a den. (Photos by R. T. Zappalorti.)

high winds) and to meet other significant needs of the target species.

Conclusions

Effective sampling for saxicolous reptiles begins with a thorough knowledge of the diversity of rocky microhabitats and the thermal and retreat requirements of the taxa likely to occur in the survey area. Capture and survey techniques include potentially habitat-destructive investigation of retreat sites and less invasive, but perhaps less effective, day and night searches and/or trapping for surface-active reptiles. Because rocky elements in many habitats are too large to be moved and too tightly packed to permit easy human access, most capture techniques require the use of extended-reach (e.g., poles, nooses) or projectile (e.g., rubber bands, dust shot) devices. Use of a combination of the techniques outlined above will likely result in a representative sample. If the diversity of local rock-living reptiles is low (e.g., in some cool temperate regions) or restricted to only one or two microhabitats (e.g., in geologically simple areas of low substrate diversity), however, one or two techniques especially suited to the expected species should suffice.

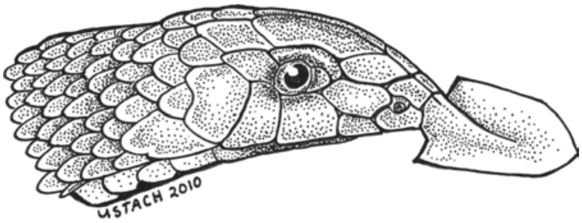
Snake Hibernacula and Communal Denning

Robert N. Reed, Cameron A. Young, and Robert T. Zappalorti

Many species of reptiles in temperate zones or at high elevations in subtropical and tropical areas avoid the thermal stresses of winter by hibernating below the frost line in underground chambers or dens called hibernacula. The reptiles may hibernate singly (Fig. 57) or aggregate in monospecific or heterospecific groups (Parker and Brown 1973; Gregory 1974). Communal denning in hibernacula is an important part of the seasonal activity cycles of some species of reptiles, especially snakes (Gregory 1984a and references therein), but also of some lizards (Congdon et al. 1979) and turtles (Carpenter 1957). Studies conducted at hibernacula can record species presence and, thus, contribute to measures of local community richness and evenness; they can also provide data on population size and demography, activity cycles, and thermal characteristics of hibernating animals (Brown and Parker 1982; Graves and Duvall 1987, 1990). Observing or capturing populations or demes at communal hibernacula allows a researcher to rigorously investigate topics such as thermal tolerances (Brown et al. 1974; Jacob and Painter 1980; Sexton and Hunt 1980; Weatherhead 1989); rates of winter mortality (Hirth 1966; Shine, LeMaster et al. 2001) and juvenile survival (Brown et al. 2007); courtship and mating behaviors (Shine et al. 2000); and movement patterns into and away from hibernacula (Reinert and Zappalorti 1988a, 1988b; Shine, Elphick et al. 2001; Wallace and Diller 2001); as well as to compare results among species (Parker and Brown 1973).

We define a *hibernaculum* (or den) as any retreat protected from subfreezing temperatures, in which reptiles spend the winter. Typically, hibernacula are not created by the target reptile, so determining occupancy of a chamber is more important than simply locating it. Consider a tortoise burrow or a coyote scat; these items provide definitive evidence that a tortoise or coyote, respectively, was present at the site at some point. A hole in the ground, in contrast, may or may not be (or have been) a snake hibernaculum. Consequently, determining occupancy of a potential hibernaculum is vital. This is typically accomplished by observing reptiles directly during ingress or egress, with remote-viewing devices, or via other means (e.g., burrow cameras).

Natural chambers that could be used as hibernacula are found in a variety of places and habitats, including rock piles (Parker and Brown 1973), sinkholes (Gregory 1974; Shine, Elphick et al. 2001), hollow trees (Kauffeld 1957), holes left by rotting tree stumps (Viitanen 1967), caves (Drda 1968; Sexton and Hunt 1980), crevices in shale (Bothner 1963) or limestone (Shine et al. 2000), ant mounds (Criddle 1937; Carpenter 1953), abandoned rodent burrows (Cohen 1948; Carpenter 1953; Viitanen 1967; Plummer 2002), and the burrows of prairie dogs (Klauber 1972; Holycross 1995), foxes (Zappalorti et al. 1983), skunks (Zappalorti et al. 1983), gopher tortoises (Moler 1992), and crayfish (Carpenter 1953; Kingsbury and Coppola 2000). Most reptile species use suitable natural crevices or openings as overwintering sites (Woodbury 1951; Viitanen 1967), although some species of snakes can excavate their own burrows (Platt 1969; Carpenter 1982; Burger et al. 1988). Reptiles can also use spaces in or under human-made structures such as railroad beds and ties (Zappalorti and Reinert 1994), old wells (Brown et al. 1974), abandoned dump sites, abandoned mines (A. T. Holycross, pers. comm.), and buried debris



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from land-clearing operations (Zappalorti and Reinert 1994), as hibernacula. It is also possible to construct artificial dens in suitable areas as conservation measures (Zappalorti and Reinert 1994; Showler et al. 2005) or specifically for observational studies of hibernating snakes (Gillingham and Carpenter 1978).

Only a small subset of this wide range of hibernaculum types is communal, containing large numbers of individuals. Despite their comparative rarity, communal hibernacula are of particular ecological and conservation interest, especially because they are typically used year after year by the same individuals (Woodbury and Hansen 1950; Brown 2008). Sampling the snakes in hibernacula can provide a demographic characterization of a population that approaches one obtained from a complete census. In addition, locating such hibernacula allows one to delimit critical winter range for species of concern for potential protection. Communal dens typically occur at high latitudes or high elevations where winter refugia that allow snakes or other reptiles to avoid freezing temperatures are rare. In the northeastern United States, Timber Rattlesnakes (*Crotalus horridus*) hibernate communally on south-facing slopes with abundant rock outcrops, where deep fissures lead to below-ground crevices (Galligan and Dunson 1979; Brown 1993). In Manitoba, Canada, garter snakes congregate below the frost line in huge numbers (in excess of 50,000 snakes) in large limestone sinkholes and caverns (Gregory 1974; Shine, Elphick et al. 2001). In western portions of North America, multispecies communal dens can be found on southeast facing slopes at high elevations (Parker and Brown 1980). Unfortunately, in various areas snakes that occupy communal dens are persecuted or harvested, especially rattlesnakes. Typically, the snakes are killed for "sport" (e.g., at "rattlesnake round-up" events), occasionally for food, or out of fear due to popular misconceptions about the human health hazards that these species pose (Brown 1993; Fitzgerald and Painter 2000).

Finding and Sampling at Hibernacula

In the following paragraphs, we discuss various tools that are useful for both detecting hibernacula and sampling any reptiles that occupy them. That the majority of studies of communally denning reptiles have focused on snakes is reflected in our recommendations for locating, monitoring, and sampling at hibernacula. A greater proportion of a local population than is typical of many reptile studies may be captured at hibernacula, especially at communal hibernacula used by all age classes. This does not mean, however, that sampling at hibernacula will necessarily produce a reliable census of a population. Any of the numerous typical analytical problems associated with estimating population sizes of reptiles may apply (e.g., age-, stage-, or sex-based variation in detection probability, observer bias, temporal variability; see Chapter 15, "Population Size and Demographics," for a discussion of these issues).

REMOTE SENSING

Aerial photography, infrared photography, and light detection and ranging (LIDAR) are remote-sensing methods that can be used to locate potential denning sites. In addition, geographic information system (GIS) technologies can be used to identify or analyze various features of a den site and surrounding areas, including land use, habitat type and characteristics, developments, wetland and water bodies, and topography. Dens located through these methods can then be ground-truthed and investigated by specialized surveys (described below) at these sites to ascertain whether potential hibernacula are occupied and to identify species use of the den.

PEDESTRIAN SURVEYS

Pedestrian surveys are often used to locate hibernacula (Brown and Parker 1976a; Reinert 1992; Zappalorti and Reinert 1994; Prior and Weatherhead 1996; Kingsbury and Coppola 2000). In pedestrian surveys investigators walk slowly through potential denning habitats (as described above) looking for snakes; a survey can be time- or area-constrained, or opportunistic (see "Visual Encounter Surveys," in Chapter 13). Such surveys can have high observer bias and therefore should be carried out by qualified biologists with target-species experience. These searches should be scheduled during appropriate seasons, times of day, and weather conditions, as snakes may be present at the entrance of a hibernaculum only briefly depending on temperature and other factors. Prior to initiating fieldwork, investigators should thoroughly review publications on the natural history of the target species to determine the appropriate temporal search window.

RADIOTELEMETRY

Radiotelemetry is one of the most productive ways of locating den sites, as snakes implanted with radiotransmitters during summer months can be followed to hibernacula in the fall (Reinert and Zappalorti 1988b). However, radiotelemetry requires expensive equipment and extensive personnel time. Once a hibernaculum is found, investigators can apply various capture techniques at the den to determine species composition, individual numbers, and occupancy rates.

VISUAL MONITORING

Known den sites can be visited during the appropriate temporal windows to detect snake occupancy visually. Investigators should ensure that repeated visits to a hibernaculum do not alter the behavior of the inhabitants or damage the den site (Brown 1993). For example, investigators visually monitoring a historic den site should hide in a blind placed to minimize disturbance and alteration of snake behavior. Visual monitoring requires significant time and personnel but can reveal the current status of a hibernaculum and provide accurate data on species and numbers of individuals present and on activity patterns. Biologists with limited target-species experience may not be qualified to carry out visual monitoring, because of the cryptic nature and camouflage of many snake species as well as observer biases that are difficult to quantify (but see Rodda 1993). If the only information required is confirmation of occupancy, burrow cameras may be used to investigate the interior of a hibernaculum, although such surveys may also have biases (Smith et al. 2005).

CONFINEMENT TRAPS

Confinement traps can be used with known or suspected hibernacula that are small, such as rodent burrows, crayfish burrows, or ant mounds, and that are not likely to contain many snakes (Carpenter 1953). These traps confine snakes exiting hibernacula to small arenas so that they can be differentiated from snakes entering the hibernacula. A series of fences and one-way funnel traps are constructed as follows: (1) The entire entrance hole or hole complex is surrounded by a cone of small-mesh hardware cloth or equivalent. The cone is placed over the entrance hole and the large end of the cone is buried at least 10 cm into the ground so as to prevent ingress or egress. (2) The trap is enclosed with an exclusion fence placed approximately 1 m from the cone, with several small one-way entrance funnels at ground level to allow entry from the exterior to the arena between the exclusion fence and the confinement cone. Snakes returning to the hibernaculum circle the exclusion fence and enter through the funnels, but the inner cone blocks ingress into the hibernaculum. The combination of the two confinement areas allows investigators to distinguish between snakes exiting the hole and snakes attempting to enter to hole. Adding a one-way exit flap (e.g., Rodda, Fritts, Clark et al. 1999) at the level of the hole but inside the internal cone could potentially reduce the number of exiting snakes that reenter the hole and escape detection, but to our knowledge this type of modification has not been field tested. Although infrequently used in recent times, this type of trap could be very effective for determining use of a known or suspected small hibernaculum. However, small confinement traps are not appropriate for large denning areas with potentially huge numbers of snakes; for such large dens, drift fences are more practical.

DRIFT FENCES AND PITFALL TRAPS

Drift fences can be used to catch snakes either entering or leaving a den. Dens surrounded with drift fences in the late winter facilitate capture of snakes emerging from hibernation (Brown and Parker 1982; Wallace and Diller 2001); in the fall and spring, they intercept snakes moving into and out of the den (Brown and Parker 1976a). Installing a drift fence around the entirety of a large den or den complex is labor intensive, but once the fence is established, it creates an opportunity to collect information on the entire denning population. Drift fences are typically constructed of aluminum flashing material, commercial silt fencing, or small-mesh hardware cloth. For studies that use drift fences and confinement cones, one assumes that no animals escape detection or climb over or burrow under the fence. These assumptions can be validated if the fence is properly installed and a mark-recapture protocol is employed. Drift fences should be buried to a depth of at least 10 cm and should have enough pitfall or intercept traps to capture most animals on both sides of the fence. Fence height will be determined by the size and habits of the target species and goals of the study. For example, a fence designed to contain snakes of large arboreal species will be much higher than one intended to contain small fossorial species. Folding over several centimeters at the top margin of the fence to form an overhanging barrier will often prevent snakes from climbing over a low fence. Additional information on traps and drift fences can be found in "Funnel Traps, Pitfall Traps, and Drift Fences," in Chapter 5, and under "Pitfall-Trap Surveys," in Chapter 13.

Several different types of traps, each with its own advantages and disadvantages, can be placed along drift fences to capture reptiles. Round funnel traps made of window screening are typically difficult to fit flush against a fence but can be bent and adjusted to fit around rocks and other features of many sites. In flat, even terrain, box traps are generally superior to round funnel traps. The former can be placed on both sides of a fence to determine ingress and egress of snakes. Another option is to cut a hole in a fence and place a box trap on one side; this setup provides a dark ingress/egress opening that can be attractive to snakes moving along the fence. A one-way door or flap installed on a box-trap funnel will decrease the number of snakes escaping (Rodda, Fritts, Clark et al. 1999). Both funnel traps and box traps should be checked at least daily and before any severe (extremely cold or hot) weather.

Another alternative is to place coverboards (Grant et al. 1992), paving blocks (Webb and Shine 2000), or similar objects along a fence to provide artificial cover or basking platforms for snakes entering or exiting a den. Coverboards do not have to be checked daily because they do not confine the snake inside a trap. Therefore, if a drift fence is monitored irregularly, coverboards should be used instead of funnel or box traps (see "Sampling with Artificial Cover," in Chapter 13).

If a single discrete entrance to a den has been identified, it may be possible to attach a large hose or pipe (rubber, PVC, or plastic) to the entrance with duct tape or other adhesive, directing emerging snakes into a corral or large trap with a one-way door (Klauber 1972). As with other confinement traps, these should be checked daily and when any extreme temperature conditions are expected. Additional information on traps and trapping is provided in Chapter 5, "Finding and Capturing Reptiles."

Monitoring Techniques

Monitoring and sampling at hibernation sites are most easily carried out in the early spring, before emergence begins, especially on warm days (>10°C), when basking may occur (Vetas 1951; Jacob and Painter 1980; Sexton and Hunt 1980). Monitoring should continue until all snakes have exited the den. As temperatures increase, traps should be checked daily to ensure that dates of emergence are recorded accurately and to prevent mortality from unexpected extreme temperatures. Fall sampling should begin when daily minimum temperatures fall below the preferred activity temperatures of the study organisms.

Passive Integrated Transponders (PIT tags; Gibbons and Andrews 2004), radioactive markers, and fluorescent dyes can all be used to monitor activity at a snake den. For example, automated systems can record snakes that have been captured and implanted with PIT tags (see "Permanent and Temporary Tags," in Chapter 9) whenever they enter or leave a

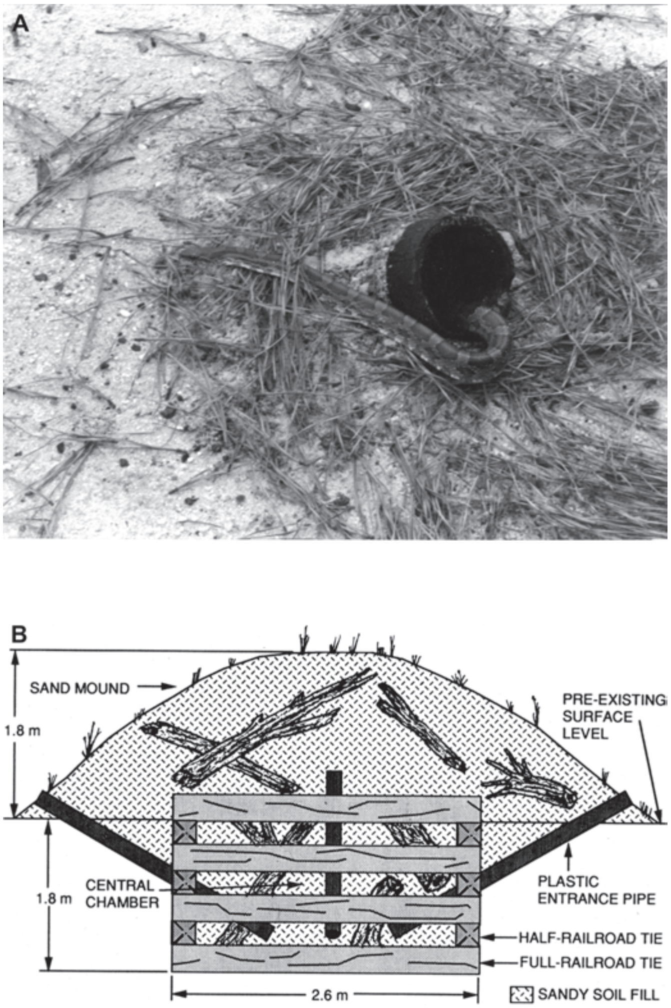

FIGURE 58 (A) Red Cornsnake (*Pantherophis guttatus*) going into the above-ground entrance to an artificial hibernaculum constructed as a conservation measure. This entrance is one of the terminal ends of the plastic entrance pipes shown in the diagram in (B) below. (B) Schematic drawing of an artificial hibernaculum constructed in a sandy substrate, so as to create multiple below-ground chambers accessible to snakes during winter. (From Zappalorti and Reinert 1994; © Society for the Study of Amphibians and Reptiles, reprinted with permission.)

den. The scanners must be placed at a natural or constructed "bottleneck" through which all individuals must pass. Because the presence of nearby metallic objects can interfere with the scanning process, any fence or housing constructed around the bottleneck should be made of wood. Automated PIT-tag systems can be programmed to record each PIT tag only once or multiple times at set intervals. Assumptions of this method are that every PIT tag is read, no PIT tag fails, and that no alternative entrances to the den exist. Automated PIT-tag systems were used successfully to record egress and ingress of approximately 350 rattlesnakes at a hibernaculum in an abandoned mine in New Mexico (A. Holycross, pers. comm.).

Lizards in hibernacula have been studied using radioactive markers (Grenot et al. 2000), but we do not recommend their use, because of potential detrimental effects such as tail loss. Fluorescent dye powder can be placed at a den entrance so that snakes move through it when entering or exiting the den. As an animal crosses the powder and crawls into the surrounding habitat, it leaves a trail that can be tracked with a field-portable ultraviolet light. However, the powder can be disrupted by wind and rain; it should be used only under optimal conditions, and the animals should be monitored frequently.

Artificial Dens

Artificial dens can be created to improve snake habitats where winter den sites are limited. Zappalorti and Reinert (1994) provided detailed plans for building a large artificial den from railroad ties, perforated PVC pipe, plastic sheeting, stumps, logs, and branches (Fig. 58). Nine species of snakes in the New Jersey Pine Barrens have occupied their artificial dens, which may be a useful conservation tool in some areas (Zappalorti and Reinert 1994). In South Carolina, artificial stump holes have been created to mitigate impacts of development, forestry practices, and stump removal on Eastern Diamond-backed Rattlesnakes (*Crotalus adamanteus*; H. Clamp, pers. comm.). Sewer junction boxes to which several 2- to 3-m-long, flexible, corrugated drainage pipes are attached are buried so that the top of the box lies approximately 1 m below the ground surface. The entrances of the pipes are only partially buried, to simulate stump-hole root channels. Research to determine the effectiveness of this latter type of artificial hibernaculum is needed.

Special Considerations

Several factors must be considered when planning surveys and monitoring programs for snake dens. Investigators designing or implementing visual surveys of known or new den sites must incorporate mechanisms to account for bias among observers with different search images, abilities to concentrate, experience, or knowledge of the target species (Rodda 1993). Observer biases should be studied to determine the effectiveness and characteristics of qualified biologists conducting visual studies at hibernacula.

Hibernacula are potentially limiting resources for many snake species (Parker and Brown 1973; Burger et al. 1988). Populations of many species have declined because of the loss of hibernacula to human development and/or the persecution of snakes at den sites (Klauber 1972; Parker and Brown 1973; Galligan and Dunson 1979; Gregory 1984a; Zappalorti and Reinert 1994). Locating hibernacula and monitoring communally denning populations of reptiles can provide information about many aspects of the target species' natural histories and can have important conservation implications. Exact locations of dens of sensitive, threatened, or endangered species should never be casually disclosed and should be reported only in conjunction with legitimate research and conservation efforts (Brown 1993).

Arboreal Reptiles: Tree-Trunk and Canopy-Dwelling Species

Indraneil Das

Sampling invertebrates from plants has been described as difficult, relative to sampling them from the ground or air, a generalization that is also true for sampling reptiles. Factors that impede effective sampling include the heterogeneous and continuous changing nature of plant-generated habitats (Southwood and Henderson 2000, p. 148), as well as their height. Indeed, arboreal habitats, which I define here as vegetation 2 m tall or taller (and, therefore effectively out of the reach of the average observer), are arguably the most difficult to sample. Rain forest trees can tower 30 m or more above the observer, with emergent trees in some areas reaching 50 m, and they have complex canopies (the upper levels of a forest). Many activities of arboreal species take place off the ground, on or in the forest canopy (where primary production takes place). Canopies are physically and biologically the most active part of the forest, and the architectural complexity of such habitats is attributed in part to the high faunal species richness in rain forests. Even when the canopy is accessible, collecting a reptile manually and inspecting the foliage with which it is associated are not always possible and depend considerably on the skills and experience of the collector. Consequently, comparing sample sets is difficult.

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In recent years, estimates of the number of species inhabiting the earth have increased dramatically (May 1988, 1990; Stork 1993). Given that the estimates were extrapolated from counts of invertebrates, chiefly coleopterans, from the rainforest canopy (Erwin 1997), it is surprising that relatively little work has been done on canopy and other arboreal reptile communities. Sampling of amphibians from the mid-canopy of forests in the central highlands of Sri Lanka, for example, has recently led to quadrupling of the known fauna (Pethiyagoda and Manamendra-Arachchi 1998). Two groups of reptiles, lizards, and snakes, form a significant part of the arboreal reptile fauna. A single turtle, the Indo-Chinese *Platysternon megacephalum,* is reported to climb trees occasionally, possibly low tree trunks, to search for insects or to bask; I do not consider it here.

Arboreal reptiles may be visible to an observer as they expose themselves on trunks, branches, or surfaces of leaves, or they may be concealed under cover of leaves, flowers, or fruits; under loose bark; or in recesses of the trunk or branches. In the latter case, claw marks, smoothed entrance holes, or shed skin may betray their presence. Sampling protocols need to encompass the enormous variety of habitats and microhabitats used by arboreal reptiles. In general, our present knowledge of the ecology and systematics of many arboreal reptile groups is rudimentary. It would not be surprising to learn that some species categorized as rare or threatened are relatively common in the canopy. Indeed, groups typically thought to be terrestrial or even fossorial have been found in numbers in arboreal situations (Rossi and Feldner 1993; Das and Wallach 1998). Collections of canopy-inhabiting species tend to be fortuitous events, for example, when animals accidentally fall from their elevated perches or when trees are logged or fall during storms. In tropical areas, it is likely that many arboreal species of reptiles remain unknown to science.

Methodological constraints are a major impediment to the growth of our knowledge of arboreal herpetofaunas, for which effective survey methods are either difficult to design or expensive. Access to the high canopy, visibility, and access to the site itself are difficult or sometimes impossible without special equipment (Raxworthy 1988). In this account, I review the various techniques employed to sample reptiles occupying arboreal habitats, including scansorial species from trees and other tall vegetation.

Field Methods for Surveys

CLIMBING TREES

The choice of a tree to climb is important and often represents a trade-off. On one hand, old trees, clad with epiphytes, strangling figs, and rotting branches are centers of diversity for arboreal reptiles. On the other, such trees are often extremely difficult and even dangerous to climb. The potential risks of climbing trees, especially in the tropics, where biting and stinging insects and other invertebrates; snakes; plants with thorns, spines, and noxious secretions; rotting branches; and vertigo are common, can be significant. The sheer hardness of the wood in many species prevents nails from penetrating the trunk or limbs. In dipterocarp forests, lack of branches at levels below the canopy frequently makes free-climbing impossible.

When an appropriate tree is selected, developing a climbing plan is essential. Branches for support should be selected on the basis of their strengths, and positioning ropes across two branches, rather than one, enhances safety. The path upward must be scanned for all potential hazards enroute, including nests of biting or stinging arthropods and obstructing vegetation. Climbers sometimes wear head nets, gloves, and clothing made of heavy materials for protection when such pests are encountered. Regardless, free-climbing a tree is an extremely dangerous activity that, in most instances, precludes the transport of bulky field equipment or supplies. To enhance safety, individuals should be well trained in the use of climbing gear before attempting to scale a large tree, and all gear must be inspected for damage before every use.

LADDERS

Simple wooden ladders permit access to the canopy of short, stunted vegetation, such as that found in many montane forests and cloud forests (A. De Silva, pers. comm. 2000). The observer moves the ladders among various sites while trying not to disturb the population being sampled. From such elevated positions, the investigator can better detect the movement or reflected eyeshine of a reptile and collect it using one of the methods dealt with later (see “Blowguns and Shotguns” and “Laser Pointers,” below). With careful examination, the surfaces of tree trunks, especially under peeling bark and within the often dense growths of epiphytes and bird’s nest fern, sometimes yield a reptile, as do birds’ nests and holes and cracks in a trunk or large limb, where unexpected reptile groups, such as dibamids and scolecophidians (generally considered to be terrestrial) may shelter.

CANOPY WALKWAYS

These structures, also known as catwalks and aerial walkways, have now been established on six continents. One walkway, built with 5-m-long sections of aluminum ladder, 13-mm diameter, 3,000-kg test-strength polyester rope, and perforated galvanized angle irons at Bukit Lanjan, Peninsular Malaysia, in the late 1960s lasted until 1976 (Muul and Lim 1970). The walkway was essentially a transect through the canopy, supported by several anchor trees. Two ropes were fixed between two trees and sections of ladders slid out on loops of rope suspended from the main cables. When in position, the loops were tied off, and each new floor section provided the base from which the next was set. Light boards were placed over the top. Rope or steel railings can be added for safety. Selection of the climbing point is important; the location of a tree on a slope can eliminate the need for a long climb up to the canopy (one enters the tree from the slope, well above its base) although this also eliminates vertical sampling (see “Tree Towers,” below). Visual Encounter Surveys (VES; see “Visual Encounter Surveys,” in Chapter 13) on canopy walkways are identical to those conducted on the ground or along waterways and are useful for compiling species lists of an area. Diurnal species are observed basking and/or foraging; nocturnal ones may forage openly under the cover of darkness and also may bask opportunistically to increase body temperature, synthesize Vitamin D, and carry out other physiological functions. On the forest floor, individuals of day-active species emerge from their hiding places as the forest warms; in rain forests, emergence often occurs around midday. Canopy species, given the more open nature of their habitats, may become active significantly earlier.

TREE TOWERS

A significant proportion of the reptile fauna of rain forests is found off the ground, and tree towers are appropriate for ecological observations and for specimen collection. Permanent towers of wood and metal now exist in several countries, primarily for botanical studies; others serve as fire-observation posts or for collecting meteorological data (described by Mitchell 1982). These structures offer potentially unparalleled opportunities for observations of a poorly known community of reptiles. Towers are sited on gentle topography, and on tall, healthy trees. Wooden platforms at various levels, up to the emergent layer of the canopy, permit long-term observations and sampling at different vertical strata of the forest. Sampling along tree towers addresses the vertical component of herpetological communities in tall forests. As with canopy walkway surveys, tree tower VESs of a vertical reptile fauna need to be conducted during the daytime and at night. The safety of the observers must be kept in mind at all times; the potential consequences of a fall from a poorly maintained canopy walkway or tree tower or through carelessness or accident are great.

CANOPY CRANES

Canopy cranes, established in many tropical and a few temperate forests, permit *in situ* studies of canopy life. A canopy crane consists of a free-standing construction crane, with a tower, operator’s cabin, load and counterbalance jib, trolley, and hook. A suspended personnel basket (gondola) is attached to the hook to carry personnel up into the canopy. At the Smithsonian Tropical Research Institute in Panama, two construction cranes allow access to the upper forest canopy and other inaccessible reaches of a rain forest, permitting observation. The observers, up to four at a time, in addition to heavy equipment, are lifted in small gondolas and lowered at desired levels within the canopy. The crane operator is in contact with the observers via two-way radio. At the Wild River Canopy Crane Research Facility, in Washington State, it is possible to access the canopy at 87 m, using gondolas that carry up to eight persons or four persons and equipment, to a maximum load of 2,286 kg. A three-dimensional system for maneuvering the gondola through the canopy is used. Attention to local weather conditions is important, and thunderstorms and icy conditions increase risks to the observer.

CANOPY RAFTS

Gigantic rafts of helium balloons ("canopy bubbles") have been placed over canopy sites in French Guyana, Cameroon, Gabon, and Madagascar to provide access to the roof of the forest (see Hoogmoed and Avila-Pires 1990, 1991; Reagan 1995). The canopy raft (also called "radeau des cimes") consists of air-inflated beams with Aramide netting between them, which are connected by ropes and set up by a team of specialized climbers. The rafts permit observers to work and live for a few days at a time in the canopy. Air-inflatable dirigibles launched close to the raft from a launch pad covered with a plastic tarpaulin (to provide cushion) allow observers to ascend to the canopy via a single-rope technique (described by Hallé and Blanc 1990). One canopy raft established in 2001 in Masoala National Park, Madagascar, serves as an access platform permitting researchers to inventory interior valleys as well as the canopy itself. The canopy bubbles, each with a 500 m^2 platform and linked by ropes, form a network that covers ca. 2 km^2, in an area ranging from sea level to ca. 400 m ASL. The high cost of establishing a canopy raft (French Francs 6 million for the one in Madagascar in 2001) has prevented the establishment of raft networks for canopy sampling in other tropical sites.

Methods for Collecting Specimens

BLOWGUNS AND SHOTGUNS

Blowguns, typically hollow wooden implements that deliver an often poison-tipped dart, have been utilized by traditional hunter-gatherer societies throughout the world. The use of blowguns can substantially increase the number of specimens of highly arboreal but seldom-collected species of lizards such as *Draco* available for study (e.g., Inger 1983). Modern, aluminum blowguns are commercially available from hunting or sporting goods shops; these devices fire a molded plastic, stun-plug pellet or a .4-calibre metal dart, also available commercially (see also "Sling Shots and Blowguns" in Chapter 5). The range, depending on the skill of the collector, can be up to 8 m. Success is greater when shots are taken horizontally, such as from an elevated site (e.g., a ridge top or canopy walkway), rather than vertically, as from the base of a tree and up along the trunk. In general, shots need to be aimed at the body to avoid damage to the head. For studies that require examination of the reproductive tracts or diet, a head shot may be preferable. Using balls of plasticine or modeling clay as projectiles can minimize damage to specimens of delicate species; other, more robust lizards (such as large skinks, with hard osteoderms in their scales, or large agamids) are often immobilized by barbless metal-tipped darts (with plastic bases). The mortality of lizards (especially scincids) collected with stun plugs and darts is relatively low. Many diurnal, arboreal lizards can also be shot with air pistols or BB guns (see "Firearms" in Chapter 5), although mortality is higher than with blowguns (Vitt and de Carvalho 1992). As with blowguns, shots can be taken at the head region to avoid damage to the internal organs. Specimens obtained may need to be preserved promptly or stored in a box of ice or similar cool chamber. The use of guns is restricted in many countries, and it is essential that investigators consult local authorities about regulations regarding their use and obtain necessary permits before using them. Regardless of collection method used, recovery of specimens shot far from the forest floor, such as from a watch tower or canopy walkway, can be difficult.

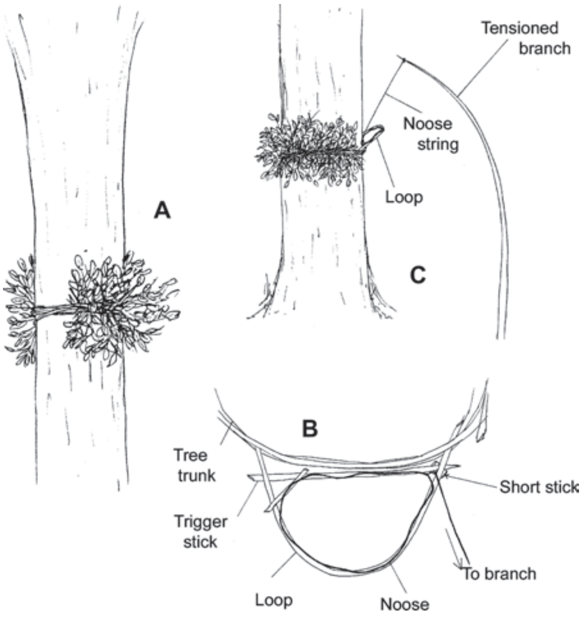

FIGURE 59 Mangmaty trap for noosing large arboreal lizards. (A) Target tree, with barrier. (B) Trigger mechanism (see detailed description under "Nooses and Baits"). (C) Tree with trap, showing angle of loop. (From Bennett et al. 2001; © Society for the Study of Amphibians and Reptiles, reprinted with permission.)

NOOSES AND BAITS

Certain large-headed lizards that permit close approach by observers (such as small varanids and iguanians) can be captured with a noose of fishing line or waxed dental floss (see "Noosing," in Chapter 5). The device can be attached to either a stout stick (in the case of a large quarry, such as a small varanid) or a telescopic (which facilitates transport) fly-fishing rod. With mealworms or similar commercially available invertebrates, fly-fishing rods can also be baited for capturing small insectivorous lizards. To reduce stress from noosing, animals to be released should be freed as soon as possible. Bennett et al. (2001) described the use of the *Mangmaty trap* (Fig. 59) for noosing large arboreal lizards (such as varanids). Materials required to make the trap, which is based on a traditional trigger-sprung trap widely used in the Philippines, include a rope or vine, 150 cm of parachute cord with a running noose tied at one end; a 30 × 16-cm loop (diameter appropriate to accommodate the lizard) made of flexible branches or vines; and two wooden sticks 5 cm in diameter, one 30 cm long (trigger stick) and the other 4 cm long (short stick). The investigator selects an appropriate tree and ties the rope or vine around the trunk, to which a wall of vegetation is attached to block off all but a 30-cm narrow path on the trunk on the side of an upward slope. The passage of the lizard through the loop puts pressure on the trigger stick, causing the small rod to detach, tightening the noose.

A second method of snaring varanids was described by Reed et al. (2000). A baited trap of wire mesh (e.g., chicken wire), to which multiple-monofilament snares are tied in a grid, is attached to a tree trunk (see Fig. 64). Hooks are tied to the wire mesh and baited with an appropriate live prey or other food. When a lizard crosses the trap, one or more of its limbs catch in the snares and pull them tight. The trap is inexpensive and easy to transport, and mortality is insignificant. Nooses and baited fishing rods can be used both from the ground, to catch lizards active low on tree trunks, as well as from tree towers and canopy walkways to capture the more arboreal species.

ADHESIVE TRAPPING

Sticky traps are an efficient method for catching reptiles, such as snakes, iguanians, scincids, and gekkonids, that move vertically along a tree trunk (Bauer and Sadlier 1992; Rodda et al. 1993). The traps are made by spreading commercially available mouse-trapping glue on hard boards, which are then attached to a tree trunk or branch. Trap placement is an important determinant of trap success; traps should be placed near basking sites, along trails, or close to observed retreats. Sticky traps can be sited in naturally shaded areas or in sunny ones (e.g., basking sites). Mortality of specimens trapped in the latter microhabitats is greater due to dehydration and, potentially, increased predation. Investigators can release captured animals by applying a few drops of vegetable (e.g., canola) oil with a paint brush. Special care needs to be taken when detaching individuals of soft-skinned species, such as gekkonids or species with tail autotomy, such as scincids. To increase capture efficiency, glue traps can be baited. Whiting (1998) used figs painted red for frugivorous cordylid lizards. For additional information on this type of trap, see “Adhesive Traps” in Chapter 5.

Unfortunately, mortality (resulting from stress, injury, and predation) is relatively high with sticky traps, as is the capture of nontarget organisms, both of which raise ethical issues. Adhesive trapping may be inappropriate for use with threatened species or for long-term field studies. Depending on the substrate used, weather conditions, and exposure, glue traps can remain effective for from 2 to 5 days. However, capture rates decrease with time, due both to depletion of vulnerable animals and deterioration of the trap (Rodda et al. 1993), and the traps are difficult to attach to the small branches and leaves that are frequented by certain species.

LASER POINTERS

Commercial laser pointers, available at office supply stores, are used in all kinds of lectures and presentations to draw attention to particular points on slides. Beams from laser pointers attract certain nocturnal, arboreal reptiles such as gekkonid lizards to the projected light, which possibly is mistaken for food or perhaps a potential competitor. From their usually high perches, gekkonid lizards can be lured toward a pre-sited assistant or a trap. This technique works well on walls of buildings as well as tree trunks, and it is more successful in the early evening than later, presumably when geckos are becoming active and are hungry.

The diode generates a narrow beam, ca. 1 mm in diameter that becomes increasingly enlarged and diffuse with distance. Some laser pointers have adjustable focusing systems, and depending on the wavelength used, 670, 650, or 635 nm (red pointer), the range in total darkness can be 300 m, 600 m, or 1,220 m, respectively. Beams from a green laser (wavelength 532 nm) have a greater capacity to hurt the retina of the human eye than the red lasers. Pointers run on 3V DC import power, most conveniently supplied by AAA or two to four LR 44 batteries (watch batteries). The energy a pointer emits is several times greater than that received by the eye when staring directly at the sun. Consequently, care must be taken not to inadvertently point the beam directly at the eyes of colleagues or study subjects. Laser beams can damage the retina, leading, in extreme cases, to loss of vision. The sale and use of certain classes of laser pointers are banned in some countries.

DRIFT FENCES

Drift fences, in conjunction with pitfall traps, have been long used to trap surface-dwelling species of reptiles and other fauna (Campbell and Christman 1982; Dodd 1991; see “Funnel Traps, Pitfall Traps, and Drift Fences,” in Chapter 5, and “Pitfall-Trap Surveys,” in Chapter 13). Vogt (1987) modified the system to trap arboreal salamanders. In principle, a drift fence is an upright surface that directs wandering reptiles (or other species) into the open mouth of a trap (usually a pitfall trap). In arboreal situations, it is often a plank that directs tree-dwelling lizards into a funnel trap. Drift fences can be constructed of any hard, smooth-sided object, including hard plastic sheets, hardware cloth, netting, metal sheets, or window screen. Traps need to be checked periodically to prevent trapped animals from starving, desiccating, or being eaten.

BAITED AND UNBAITED TRAPS

Depending on the activity and behavior of the target species, specific traps can be installed to capture arboreal reptiles. Minnow traps, which are easily manufactured (instructions are available on various websites) as well as available commercially in various sizes and materials, are especially effective. The trap consists of a cylinder with a funnel extending inward at one or both ends or on one side. Zani and Vitt (1995) used commercial minnow traps in Ecuador to capture tropidurid lizards that use tree holes as refugia. They chased lizards into a tree hole, plugging all other holes with cloth, and then placed the entrance of their minnow trap over the hole. They tied the trap securely to the branch with nylon string. Lizards emerging from the hole (the only one available) entered the minnow trap, whose entrance had been modified to accommodate the head dimensions of the largest males in the target group. Rodda, Fritts, Clark et al. (1999) also used minnow traps to capture *Boiga irregularis,* a snake pest, for the purpose of control. Capture rates were enhanced through the use of mouse baits. Rodda and Nishimura (1999) reviewed the effectiveness of a variety of trap types. Several traps were designed to discourage retreat of entering snakes, including "metal whisker" traps (in which the mesh screen around the entrance hole is frayed), "split-cone" traps (in which the plastic around each entrance hole is slit radially many times [>12]), and "flexible squeeze" traps (in which the entrances are comprised of folds of window screening or other mesh pieces that meet along a linear midline). Traps may be baited with an appropriate live prey or other food or left unbaited. Most traps used to sample arboreal reptile populations work for snakes and large lizards (such as varanids). Traps, however, are prey specific, and not all arboreal reptiles can be trapped.

CANOPY FOGGING

Arboreal invertebrates are widely sampled by fogging the canopy of a tree with biodegradable pesticides of the pyrethrin group, which cause enhanced activity, leading invertebrates to fall off the tree (Southwood and Henderson 2000, p. 156). Pyrethrins have no long-term effects on development or reproduction in invertebrates (Paarmann and Kerck 1997), but their effect in reptiles remains unknown. The arthropods are collected from a plastic sheet placed under the tree either on the ground or on a raised metal frame with short legs. In rain forests, a mist-blower or fogger that has been hoisted into the canopy using a rope and pulley system produces a fine spray of insecticides that drifts and penetrates the canopy. The release of the insecticide is controlled from the ground by a radio transmitter that operates a servo unit on the fogger, opening and closing the insecticide-release valve (see Stork and Hammond 1997). In the Danum Valley, Sabah, in northern Borneo, where extensive canopy-fogging studies have been carried out, arboreal skinks belonging to two genera (*Sphenomorphus* and *Lipinia*) have been taken as incidental catch (ID, unpubl. data). Reptile species that can evade the chemicals by retreating into sheltered locations, such as cracks and holes within the trunk or branches, are not represented in the samples. Limitations of fogging include its dependence on calm weather (such as at daybreak) and the lack of information on the effects on reptiles of the chemicals typically used (specific for arthropods). Additional studies of canopy fogging as a method for sampling arboreal reptiles are needed. Development of site-specific methods for sampling the canopy herpetofauna should be a priority, and canopy-fogging protocols for collecting invertebrates in tropical rain forests should incorporate techniques for sampling arboreal reptiles.

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Swamp-Dwelling Crocodilians

William E. Magnusson

Swamps are ubiquitous but hard-to-define habitats. Open marshes, floating grass mats over lakes, flooded shrublands, and mangrove forests are all considered swamps. In general, if it is wet and hard to survey, it is a swamp. Swamps border most rivers and lakes, and most swamps have rivers or lakes that drain them. Therefore, most species of crocodilian inhabit swamps to some extent.

The swamp obviously becomes a problem to the surveyor when the target species is inaccessible because of difficulties of moving through thick vegetation. However, swamps are often the homes of nesting female crocodilians (Joanen and McNease 1987), which are demographically the most important segment of the population, even though most males and juveniles may live in other habitats. Estuarine Crocodiles, *Crocodylus porosus,* including adult females, spend most of their lives in estuarine habitats. However, in many areas, the only available nesting sites are found in small freshwater swamps (Magnusson et al. 1980). Density may be a misleading indication of wildlife habitat quality (Van Horne 1983). This is especially important for crocodilians because individuals excluded from preferred areas by the territorial behavior of dominants may congregate in suboptimal habitats (Messel, Vorlicek, Wells, and Green 1981).

Surveys in Adjacent Habitats

Investigators have rarely surveyed flooded forests because trees cannot persist in areas that are permanently flooded. It is usually easier to carry out surveys when tidal or seasonal decreases in water levels concentrate crocodilians in smaller areas of open water where they can be detected by spotlight surveys (see "Finding Crocodilians," under "Finding, Counting, and Catching Crocodiles," in Chapter 5). The assumption that all individuals leave previously flooded forest at low water has not been tested for most species. *Osteolaemus tetraspis* appear to remain in terrestrial burrows at low water (Riley and Huchzermeyer 1999), and *Paleosuchus trigonatus,* while not strictly a swamp species, spend much of their time in burrows in areas of forest that do not flood (Magnusson and Lima 1991). Surveys for these species require a lot of walking, as well as knowledge of the behavior of the species that can be obtained only with radio telemetry.

As with any survey, the appropriate sampling method depends on the data that are needed (Webb and Smith 1987). However, interpretation of the results is unlikely to be possible unless based on accurate maps of the swamps and other aquatic habitats surveyed. Therefore, considerable effort should be devoted to analysis of aerial photographs and satellite images of those areas. Evidence of crocodilians around a swamp is good evidence that crocodilians use the swamp. Therefore, spotlight counting in surrounding open water may be useful in broad-scale surveys to determine the presence or absence of crocodilians in the region. If a large sample of the observed crocodilians can be captured, analyses of the population size structure and sex ratio will provide some indication of the role of a swamp in the population dynamics of the species. If mainly adult males and juveniles are captured, the swamp is probably functioning as a source and the open water areas as a sink for the population.

Even if the surrounding areas contain a balanced sex ratio, the swamp may be important as a refuge from hunters. Considerable evidence in the wildlife and fisheries literature indicates that management is more likely to be successful when hunter access to a source population is reduced (e.g., Allison et al. 1998; Novaro et al. 1999). Swamp crocodilians may also be more resistant to hunting because of a decreased frequency of social interactions with larger individuals of the same species

(Magnusson 1986). More detailed analysis of the importance of swamps to population dynamics of crocodilians will require information from within the swamps, and the time and money required for surveys will escalate.

Mark-Resight Techniques

Relatively open swamps can often be surveyed by boat or aircraft. Bayliss (1987) marked crocodiles (*Crocodylus porosus*) with plastic cattle tags attached to detachable barbed metal shafts that were driven into the neck skin with a harpoon pole. He then employed mark-resight techniques to estimate the population size in several habitats in northern Australia. The proportion of marked animals resighted was relatively low in mangrove-lined creeks. Bayliss (1987) also carried out daytime helicopter surveys of mangrove habitat in areas where crocodile densities had been estimated previously by mark-resight techniques. He found that the helicopter surveys detected only a small proportion of the population known to be present in mangrove-lined creeks. Mourão, Bayliss et al. (1994) marked Yacare Caimans (*Caiman yacare*) in vegetation-covered lakes of the Pantanal with white paint and resighted them from an ultralight aircraft. They determined that only about 2 percent of the caimans in the lakes were visible from the air. Mark-resight techniques are too expensive to apply on a large scale and are used mainly to calibrate other techniques for use in similar habitats.

Radiotelemetry

Radiotelemetry studies may aid in the interpretation of results of spotlight and other surveys of small swamps. If an investigator can capture a reasonable sample (e.g., >10 individuals) of each demographic segment (adult males, adult females, and juveniles) in the swamp and attach a transmitter to each individual, then she or he can use subsequent locations of the animals taken from the radio signals to estimate the proportion of time each group spends outside the swamp (e.g., Joanen and McNease 1970). Such information provides a basis for calibration of spotlight counts in aquatic habitats adjacent to the swamp. Radiotelemetry is probably the only secure means of determining habitat partitioning among size and sex classes. Radio telemetry also may indicate the proportion of time that forest animals spend in burrows away from streams (Magnusson and Lima 1991). The easiest way to attach radio transmitters to crocodilians is to sew them onto the tail scutes (Muñoz and Thorbjarnarson 2000; Campos et al. 2006); unfortunately, the exposed radios can catch on the thick vegetation in swamps, pulling out the sutures and causing the radio to detach from the animal. An alternative is to implant radios in the abdominal cavity (Magnusson and Lima 1991; Campos et al. 2006). This is more time consuming and stressful for the animal but has the added advantage that data can be obtained on body temperatures (Campos et al. 2005).

Hand capture of crocodilians in swamps is difficult. However, various types of traps used in open water (e.g., Hutton et al. 1987; Walsh 1987; Mazzotti and Brandt 1988) also work in swamps. Small (total length <1 m) crocodilians are easily captured in baited turtle traps, but larger individuals may tangle their teeth in the bottom of the trap and drown. The time and costs associated with capturing crocodilians in swamps is usually worthwhile only if the individuals will be equipped with radios and monitored intensively.

Diurnal and Nocturnal Surveys

Spotlight surveys from open water are rarely useful in swamps because the light penetrates only a short distance into thick vegetation and most animals are likely to be missed. At the same time, surveys on foot are usually too laborious to be effective over large areas. Airboats and marsh buggies, which can travel over thick reeds and provide access to the center of open marshes, have been used in many studies of American Alligators, *Alligator mississippiensis* (e.g., Joanen 1969; Joanen and McNease 1979). However, these vehicles destroy vegetation and wildlife, and their use in most parks and reserves is now prohibited or severely restricted. Airboats and marsh buggies would be especially appropriate for seasonally flooded swamps, such as the Pantanal and the Llanos, where the periods of high water are too short for the production of luxuriant aquatic vegetation. Nonetheless, their high costs and maintenance requirements have so far restricted their use outside of North America. Nocturnal helicopter surveys may provide accurate counts in some swamps (Graham 1977), but the high cost and low safety margin of helicopters preclude their use in most situations.

Mourão et al. (2000) carried out daytime aerial surveys of caimans from small planes in the seasonally flooded swamps of the Brazilian Pantanal. They were able to count individuals easily only when low water levels caused them to concentrate in open areas. Most of the variation in numbers of individuals observed was related to the level of flooding and, therefore, the degree of animal dispersion and amount of aquatic vegetation lining residual waterbodies at the time of the survey. Similar problems have been reported for ground surveys of caimans (Campos et al. 1994) and boat surveys of alligators (Woodward and Marion 1979), caimans (Da Silveira et al. 2008), and crocodiles (Montague 1983; Jenkins and Forbes 1985; Cherkiss et al. 2006) in swamps and wetlands.

Artifacts as Indices

In the absence of direct counts of individuals, artifacts may be used as indices of population size. Riley and Huchzermeyer (1999) concluded that pools and burrows indicated the presence of *Osteolaemus tetraspis* and guessed the number of individuals in burrows to estimate population sizes. Track surveys on mud banks in mangrove forests can also be used as indices of population size or to determine the size structure of segments of the population (e.g., Wilkinson and Rice 1996). However, nests are the artifacts most widely used to monitor crocodilians in swamps.

All crocodilians that nest in swamps make large, long-lasting mound nests (Greer 1970) that can often be detected from ultralight aircraft (e.g., Campos 1993), helicopters (e.g., Webb, Whitehead, and Manolis 1987; Campos and Mourão 1995), or fixed-wing aircraft (e.g., Magnusson et al. 1980). The number of nests detected can be used directly as an index of the number of breeding females (e.g., Hollands 1987); alternatively, counts of nests detected by independent observers can be manipulated statistically to estimate the total number of nests present (e.g., Magnusson et al. 1978; Mourão, Campos, and Coutinho 1994; Mourão and Magnusson 1997). Regular monitoring will reveal any extreme drop in nest density that could indicate a reduction in population size.

Chabreck (1966) presented a formula for estimating the number of American Alligators in an area based on the average number of nests counted. However, nest densities usually fluctuate widely among years, depending on climatic and other environmental factors (e.g., Hayes-Odum and Jones 1993; Campos and Magnusson 1995), so short-term monitoring is usually of limited value, and it is extremely difficult to relate nest numbers to numbers of breeding females or total population size. The State of Florida conducted one of the most detailed (and expensive) population studies of any crocodilian species on American Alligators in Orange Lake (Hines and Abercrombie 1987). Based on the study results, the Florida Game and Freshwater Fish Commission subsequently allowed hunters to take a large proportion of the adult females estimated to be in the lake. After 4 years of hunting, no effect on the number of females nesting around the lake was apparent, a result that was not biologically feasible if the original density estimates were correct (Hines and Abercrombie 1987). Attempts to estimate the absolute densities of crocodilians in swamps are, as in other habitats, unlikely to be cost effective. Careful monitoring of relative densities is much more likely to be of value for adaptive management (Bayliss 1987).

Acknowledgments

This chapter contains few original ideas, and I thank Grahame Webb, Peter Bayliss, Ted Joanen, Ronis da Silveira, Zilca Campos and Guilherme Mourão for what they have taught me about swamp crocodiles. The paper was written while I was a recipient of a Bolsa de Produtividade scholarship from CNPq.

Detecting and Capturing Turtles in Freshwater Habitats

Richard C. Vogt

On Land

BY HAND

The most primitive methods of capturing both freshwater and terrestrial turtles is by hand after stumbling upon them in the leaf litter of the forest understory (e.g., *Terrapene carolina, Kinosternon leucostomum, Geochelone* spp.) or encountering them as they cross a road. Many aquatic and terrestrial species cross roads during migrations to nesting or hibernation sites, during spring or post-hatching dispersal, or after departure from drying ponds. Road-killed turtles can document valuable locality data as well as estimates of population size based on the quantity of road kills. Researchers studying terrestrial turtles often walk systematically through favorable habitats looking for turtles or their sign.

SIGN

Sign includes scats, tracks, burrows, partially eaten vegetation (*Opuntia* fruits, leaves with bite marks), fresh nests (or ones that have been preyed on), and turtle trails. With an intimate knowledge of turtle ecology and behavior as well as a good search image, investigators can look for the correct type of sign in the most appropriate places for the season and weather conditions. Legler (1960b) systematically searched for Ornate Box Turtles (*Terrapene ornata*) under shrubs and mulberry trees, along trails, and along unplanned drift fences (e.g., stone fences in Kansas), as well as looking for cattle dung that they had torn apart to locate the contained dung beetles. He also systematically rode through these areas on horseback because it was easier to see the turtles from higher up and because the turtles were accustomed to horses and did not spook as easily as they did in response to humans.

TRAPS AND TOOLS

Researchers have captured *Gopherus* tortoises around active burrows using hooks (Woodbury and Hardy 1948), small-mammal live traps, pitfall traps at burrow entrances, and drift fences with pitfall traps or funnel traps (Plummer 1979). They also may dig an individual from a burrow. More-creative methods of turtle capture include (1) releasing a male tortoise into a burrow (if a resident male is present he will usually come charging out after the other male), and (2) installing a Plexiglas door at the burrow entrance that permits the tortoise to exit but not reenter. Indigenous people of the Amazon rain forest collect *Geochelone* spp. in large pitfall traps. They kill a large mammal such as a paca and hang it above a 1- to 2-m-deep straight-sided hole. As the carcass putrefies, the odor attracts tortoises (and other animals) of all sizes from the surrounding area; while searching for the carcass, they fall into the hole. The pits are checked after a few weeks.

DOGS

The most efficient way to collect terrestrial turtles of any kind in any habitat is with trained dogs, whose olfactory senses far exceed those of their handlers. More than 90 percent of 3,832 box turtles collected by Schwartz and Schwartz (1974) were found by their Labrador retrievers. A dog trained by commercial turtle hunters helped us to find *Kinosternon leucostomum* when they were estivating in the forest (Morales-Verdeja and Vogt 1997). The dog looked for turtles in the leaf litter, under boulders and fallen trees, and under water. Breeds of dogs that are best for turtle hunting have a keen sense of smell, are close to the ground, and have been trained from a young age to hunt turtles; generalized hunting dogs are easily distracted by the scent of other game (D. Moskovits, pers. comm.).

DRIFT FENCES

Drift fences, often combined with pitfall traps and funnel traps (see “Funnel Traps, Pitfall Traps, and Drift Fences,” in Chapter 5), are effective for sampling populations of terrestrial turtles as well as freshwater turtles migrating on land to or from nesting grounds or leaving a drying pond. Drift fences work only when the animals are moving, so collectors must have some knowledge of the turtles’ activity patterns. Sexton (1959a) increased his success at collecting *Chrysemys picta* by blocking the migration route to its nesting ground with 25-cm-tall, 100-m-long barriers and slowing the turtles to his pace. Gibbons (1970) modified this technique by completely encircling ponds at the Savannah River Site (SRS, Department of Energy, South Carolina) with drift fences with pitfall traps; he managed to collect nearly all of the turtles from the ponds during their nesting or other terrestrial migrations. He used the system to catch previously captured turtles as well as newly recruited hatchlings on their return to the pond.

Drift fences must be monitored on a regular basis depending on the number of turtles moving through the area and the density of predators. Mike Pappas (pers. comm.) had so many *Emydoidea* moving along his drift fences that if the traps were not checked every few hours during peak nesting periods the buckets would fill with turtles. Predators, such as raccoons (*Procyon lotor*), also learn about drift fences and will walk along them to harvest hatchling turtles in the pitfall traps. For this reason, at least during the hatchling migration season, buckets should be fitted with screen, plastic, or metal funnels that allow hatchlings to pass through into the bucket but prevent raccoons or other predators from gaining access (Vogt and Hine 1982).

Drift fence designs for sampling squamates are discussed elsewhere (see "Pitfall-Trap Surveys," in Chapter 13). Adding turtle traps increases trapping efficiency. The best material to use for fences depends on several factors: efficiency at capturing the target species, ease of installation and maintenance, permanence, and cost. The complete-enclosure fence and buckets arrangement that Gibbons (1970) used at the Savannah River Ecology Laboratory (SREL, University of Georgia, at SRS) is a relatively maintenance-free system that lasts for decades. However, the aluminum flashing or galvanized sheet metal that he used for the drift fences is cost prohibitive for some studies, too heavy to carry into distant areas, attractive to thieves in well-populated areas (for house repairs or selling for scrap metal), and so forth. In public-access areas, aluminum flashing may even attract vandals (RCV, pers. observ.). Thick industrial plastic tarp material can be used for the drift fences instead of metal; it is inexpensive, easy to carry, and has little resale value, making it unattractive to vandals. When not in use, the plastic can be dropped to the ground, rolled, hidden (covered with leaves, sand, soil, snow, or other natural material), and employed again when desired. On the other hand, plastic has high maintenance requirements. Holes and tears caused by animals passing through the fence, falling branches, wind, and so forth have to be repaired regularly, and fences must be raised upright after being flattened by heavy rains, hail storms, or branches, or even replaced after being destroyed by one of those factors. Also, the replacement interval for plastic is much shorter than that for metal materials, which can remain in place for years, even in the tropics. Plastic or aluminum window screening can be used, but both cost more than plastic tarp material and are more difficult to hide when a fence is lowered to the ground. Researchers who work in secure areas and have the funds should use metal drift fences. Thirty meters (at least) of drift fences with pitfalls can be setup in sections to transect the overland migration routes of aquatic turtles from the water to nesting sites. In general, arrays of drift fences with pitfall traps designed for catching reptiles and amphibians are useful for recording the presence of some terrestrial turtles (e.g., *Terrapene*) in the habitat being sampled, although I am unaware of any long-term studies in which drift fences have been used for continual capture of these animals (Vogt and Hine 1982).

In Freshwater

HAND CAPTURE

Basking aquatic turtles can often be captured by hand or dipnet if approached underwater or from the rear. Investigators traveling by boat can rush basking turtles at full speed and often pluck them off a log or sweep them into a dipnet. The late Fred Cagle, a herpetologist from Tulane University, was notorious for using this technique day and night to collect *Graptemys,* which often sleep underwater on submerged branches in clear-water habitats (Cagle and Chaney 1950; J. L. Dobie, pers. comm.). Nevertheless, the technique is inappropriate for systematic sampling. In the rivers of southern Mexico, my students and I saw *Dermatemys* swimming underwater in clear-water streams, but they were too fast to catch if one was snorkeling. We were, however, able to capture them by diving off a moving boat. We used this technique day and night with a spotlight. J. M. Legler (pers. comm.) also caught most of the turtles in his Australian studies by diving for them. I have also approached *Graptemys ouachitensis* while they were surface feeding in the Mississippi River backwaters. When they submerged their heads to feed, I moved forward rapidly; when they emerged to look around, I froze. My head was camouflaged with a mat of aquatic vegetation, and only my eyes were above water. Because it takes 20 to 30 min to catch a turtle, this technique it is not very efficient.

Muddling, or *noodling* (see "Blind Capture," under "Alternative Methods for Sampling Aquatic Turtles and Squamates," below), for turtles in shallow water involves feeling for them in the mud, the nooks and crannies below logs, snags, rocks, and under overhanging banks with your hands. Such haphazard methods of capturing turtles suffice for locality documentation but are generally inappropriate for quantitative sampling. Investigators working in a closed system, such as a small pond, can perhaps capture all of the turtles present by muddling. In the Brazilian Amazon I have caught more than 40 *Podocnemis unifilis* within 2 hours in small backwater ponds during the dry season. M. A. Ewert (pers. comm.) collected thousands of turtles this way throughout the United States. Kinosternids are particularly easy to catch in this manner. An extension of muddling is *sounding*, or *poling*, in which the collector abruptly drops a wooden pole into the mud in springs or in areas of water where bubbles are surfacing. If the collector hears a hollow plunk, she or he, reaches underwater to grasp the turtle. Professional turtle trappers in Wisconsin effectively diminished populations of *Chelydra* and *Glyptemys insculpta* congregated in winter hibernation using this technique. Usually there is a hook at one end of the pole that is used to pull the turtle from the water. This technique has also been used in Mexico during the dry season to find *Staurotypus*. In the Brazilian Amazon, the hook is replaced with a spear point. The spear head, a rectangular point of steel, is quite small (2×3 mm) and is tied to the pole by a string. When the spear head strikes a turtle, it is released from the pole and impales the turtle carapace, functioning much like a harpoon. The investigator uses the string to haul in the point and turtle. This method is best suited for documenting a species' occurrence. Poling for turtles requires some basic training and a feeling for where turtles can be found.

Collectors in Mexico use a similar technique for terrestrial turtles, particularly *Kinosternon acutum* and *Rhinoclemmys areolata*. A blunt 2-penny nail is driven into a 25-mm-diameter wooden pole that is thrust horizontally through the leaf litter at the base of shrub clumps. When the nail hits a turtle, it makes a plunking sound, different from that made when hitting a rock or tree branch. We located several dozen turtles on a regular basis when using this technique. The technique is especially valuable for locating turtles during the dry season, when they do not move around. Carpenter (1955) used a similar technique to locate five species of turtles, including hibernating individuals.

BASKING TRAPS

Traps designed to capture basking individuals are effective in temperate climates for the many species of turtles that bask frequently. The simplest design is to attach plastic or metal baskets to the basking logs (Carr 1952). A collector surveys the area to be sampled with binoculars or a spotting scope to locate the preferred basking sites. He or she then attaches baskets to one side of the log below areas where turtles are accustomed to returning to the water. The lips of the baskets should be flush with the water surface so that the turtles can escape when the baskets are not attended. The collector rushes the basking log from the side opposite the baskets, the startled turtles fall into them, and the collector rapidly removes the turtles before they climb out. Brian Horne (pers. comm.) used this technique successfully when studying *Graptemys flavimaculata* on the Pascagoula River in southern Mississippi. This technique allows for dietary studies because fresh stomach contents can be collected from turtles that have not consumed trap bait (e.g., see Legler 1977; Fields et al. 2000). Also, because the turtles have not been stressed by trap confinement, blood can be analyzed for circulating hormone levels without fear of biased results caused by stress. The drawbacks of the technique are that it only works with species that bask, is time consuming, requires ideal basking sites, and in most species, apparently, is biased toward females and juveniles.

Floating basking traps consist of a square or rectangular wooden frame with a wire mesh or nylon bag attached beneath the center. Planks are angled like seesaws, from the surface of the water, over one side of the frame (which serves as a fulcrum), and toward the center of the trap; the turtles come out of the water to bask and climb higher and higher on the wooden planks until they reach the other side of the fulcrum and are dumped into the trap basket, from which they cannot escape. The seesaw plank reverts to its position in the water after the turtle slides off. Although these traps are good for long-term population studies, they are not adequate for routine sampling, because the turtles must become accustomed to the newly offered basking areas, the traps are bulky to carry around, and many turtle species do not bask.

Another active basking trap is a modification of the balchatri trap used to capture birds of prey and wading birds (Foster and Fitzgerald 1982). Hundreds of snares (slip nooses) made of monofilament fishing line are tied to pieces of galvanized chicken wire 0.5 m on a side (Fig. 64). The wire is then molded to fit onto any basking surface and nailed or tied to it, or it can be placed on sand beaches. As the turtle walks over the trap, its arms or legs get caught in the nooses, which close as the animal struggles. As with any basking trap, the investigator must know where the turtles are basking. After the trap is in place, it must be checked every few hours to keep the turtle from drowning, excess sun exposure, or being taken by birds of prey (Braid 1974). Various more complicated basking traps with multiple treadles have been invented; they are described in Lagler (1943), Breckenridge (1944, 1955), Ream and Ream (1966), Robinson and Murphy (1975), and Plummer (1979).

BASKING SURVEYS

Basking surveys can also be conducted using high-powered binoculars or spotting scopes. Obviously these types of surveys are only appropriate for species that bask at certain times of day and seasons. C. J. McCoy and I (pers. observ.) used this technique most effectively for assessing population densities of *Graptemys flavimaculata, G. nigrinoda,* and *G. oculifera*. Our data were substantiated by simultaneous trapping with Fyke nets (extensions that extend out from hoop traps that function like drift fences; see description under “Baited Hoop Nets,” below). We floated downstream in a boat or canoe, one person in the stern steering and the other in the bow with a 30X spotting scope. To standardize our results we floated rivers on sunny days for 2-h periods between 09:00 and 11:00, when turtles would most likely be basking. We were able to identify and determine the sex of adults of eight species of turtles as well as identify hatchlings to species. We also identified but did not determine the sex of two additional species. Lindeman (1997) also used this technique for censusing *Graptemys* populations in Tennessee. *Podocnemis expansa* can be surveyed by airplane at their nesting beaches in Tabuleiros, Brazil, because during their characteristically short nesting season they spending most of the daylight hours basking. Because individuals of this species are large, it may be possible for investigators to use newly designed satellite radar systems to count aggregations of basking turtles throughout the Amazon Basin without ever leaving the office!

NESTING BEACH SURVEYS

Without getting wet, buying traps, or seeing turtles, investigators can document the presence of even rare species of turtles by surveying potential nesting sites. Surveys during the nesting season, when tracks of females lead to the nest, are the most accurate. Tracks and eggs of most species can be identified at least to genus, (c.f., sympatric species of *Graptemys,* Vogt 1980a). Although nesting seasons are relatively short, species can be identified from egg fragments later in the season, after hatching or nest predation. Recently, dichotomous keys for identifying egg-shell fragments to species have been developed (R. Saumure and J. Bonin, pers. comm.). These types of nontraditional survey methods combined with trapping and basking surveys can enhance the quality of surveys for rare, threatened, or endangered species, relatively inexpensively.

BAITED HOOP TRAPS

A hoop trap is one in which a series of metal, wood, or fiberglass hoops placed at 0.5- to 1.0-m intervals are covered with nylon netting or galvanized poultry wire to form a tube. The diameter of the hoops varies according to the depth of the water where the trap will be set. A funnel entrance (small opening attached to the trap) is constructed at one (or both) end(s) of the trap using the same material. The opening of a funnel must be maintained taut so that turtles can easily force their way into the trap but have difficulty finding the opening to escape. The design of the hoop trap is about 6,000 years old (Singer 1954, as cited in Legler 1960a). In 1960, Legler simplified and standardized construction, using lightweight durable materials. The resultant *Legler Trap* has been used by researchers for more than 40 years to sample and study turtles that are attracted to bait. Fifty-cm-diameter hoops are made of 1-cm-diameter aluminum tubing, 1-m-diameter hoops of 1.5-cm tubing, and larger-diameter hoops of galvanized steel or fiberglass. Four hoops are covered with a single rectangle of 2-cm-mesh nylon fishing net to form a tube. The loose ends of the netting are inverted into the hoops at each end to form the funnel throats of the trap. The traps are kept rigid by a pair of dowel stiffeners with metal screw hooks placed on each side of the trap. These stiffeners maintain the form of the trap and, in particular, keep the throats taut, such that turtles must push their way into the trap but then have a hard time finding their way out.

Sardines in oil, canned cat food, fresh chicken parts, fish, shrimp, or other aromatic fare can be placed in a bait container made from window screen, aluminum beverage cans, or wide-mouth jars or vials. The closed container, or its lid, is punctured profusely so that the scent of the bait escapes but the bait is inaccessible. The bait containers are hung from the center of the trap. Turtles tend to stay in the trap trying to get the bait, and the natural stomach contents are not mixed with the bait. Bait should be changed daily. Traps checked at 4- to 8-h intervals usually produced a higher catch than traps checked only every 24 h. Legler (1960a) found that traps 48 cm in diameter, 83 cm long, and with a throat depth of 30 cm were the most effective at catching turtles. These traps are inexpensive to build (less than US$10 in 2008); small, light, and easy to transport and store; and easy to use. A 2- to 4-m nylon line is attached to one of the end hoops, and the trap is hurled into the water near a log, eddy, or other probable capture site. The loose end is tied to a tree, stake, or rock so that turtles or other animals in the trap cannot move away with it. An investigator can also position the trap with this line so that part of it is above water to prevent drowning of the catch. One advantage of the Legler Trap is the convenience of setting and checking it without having to get into the water.

Since Legler developed his ingenious design (Legler 1960a), it has been modified by various investigators who have substituted chicken wire, metal hoops, fiberglass hoops, and wooden hoops for Legler's original materials and attached a box at one end of the trap to hold additional turtles (see Kennet 1992). A variation developed by Iverson (1979a) is standard for catching *Kinosternon* and *Trachemys*. He used galvanized chicken wire to make his traps, which are considerably cheaper but much more cumbersome than the Legler Traps, even though the traps can be collapsed for storage and reshaped for later use. Also, otters, piranhas, and crocodiles created fewer holes in the metal traps compared to those of mesh netting. Nevertheless, I found these traps to be less effective than the standard Legler Trap at capturing turtles.

The standard hoop-trap design can be modified to trap large turtles. The most important characteristics of such traps are their lengths and throat diameters. The throats must be taut and long but with a small opening so that it is more difficult for a turtle to swim out of the trap than to swim in. Long throats keep the turtles either above the opening or below it trying to find a way out in the corners. The classic Legler Trap has throats that are 7.5 cm high and 25 cm wide. These traps are especially useful for carnivorous or omnivorous species that respond to baits, such as those in the genera *Trachemys, Kinosternon, Sternotherus, Claudius, Staurotypus, Phrynops, Trionyx, Chelydra,* and *Macrochelys*. Most herbivorous species rarely enter hoop traps unless leads (nylon fishing net with lead weights attached on the bottom edge and a float line (nylon cord with a floating nylon foam core) attached on the top, functionally a drift net) are attached in front of the traps. The leads must be the same depth as the water to be effective, that is, the turtles must not be able to swim over or below them. Instead, the turtles are herded into the traps as they try to get past the leads. The traps work on the premise that the turtles are active and hungry or sexually motivated. Trapping hungry turtles in cold water or in early spring, fall, or winter generally is not successful. *Chrysemys picta,* however, were enticed to enter traps baited with conspecifics in the early spring when these turtles were copulating but not yet feeding (Vogt 1979). A decade later, Frazer et al. (1990) independently discovered this behavior in *C. picta*. They did not, however, place their turtles in bait containers within the traps and noted that a number of the "bait turtles escaped"—a major problem with this type of trap if the throats are too short and the openings are too large.

Kennet (1992) modified the hoop-trap design to make the traps virtually escape proof. His single-throated turtle trap has an entry section with a funnel leading to the bait and a holding section from which the turtles cannot escape. The sections are joined by a rectangular tunnel of wire mesh with a one-way plastic mesh door that allows turtles to enter but not leave the holding compartment. The two-section traps are easier to position with the holding pen at least partially out of water and can be left unattended for several days without the turtles drowning or escaping. Predators, however, can be a problem.

Members of the commercial fishing industry use double-throated hoop nets of the same general design as the basic turtle trap, but their traps include seven hoops ranging from 40 to 600 cm in diameter. The long-fingered throats are attached to the second and fourth hoops, permitting the turtles to swim into the traps but making it very difficult for them to leave; because they have to force their way through the funnel mouth to enter the trap, it is almost impossible for the reverse to occur. These traps have to be set either by boat and or by walking into the water. A structure equivalent to an aquatic drift fence is often produced by attaching 15- to 30-m-long leads from the end of one trap to the end of the next. The lead is made of the same mesh-size net and extends from a float line at the surface to the bottom; a turtle cannot get above it or below it and so swims along it and ends up in a trap (Vogt 1980b). Sizes of the traps can vary depending on the depth of the water to be sampled. Turtles are not attracted to these traps by bait but merely guided into them as they try to circumvent the leads. Consequently, the traps do not target hungry carnivores and thus get a more accurate sample of the species diversity and abundance in a defined habitat. Again, the traps must be set during the season of the year when the turtles are active. Also, one must remember that some species are sit-and-wait predators, whereas others are active foragers. Nets set in front of nesting beaches are obviously going to be biased for females, although in some species males swim around more than females and are more likely to be captured. I have used this technique with great success in ponds, lakes, streams, and fast-moving rivers for all types of turtles and in all habitats from the Mississippi River of Wisconsin to the Amazon Basin in Brazil.

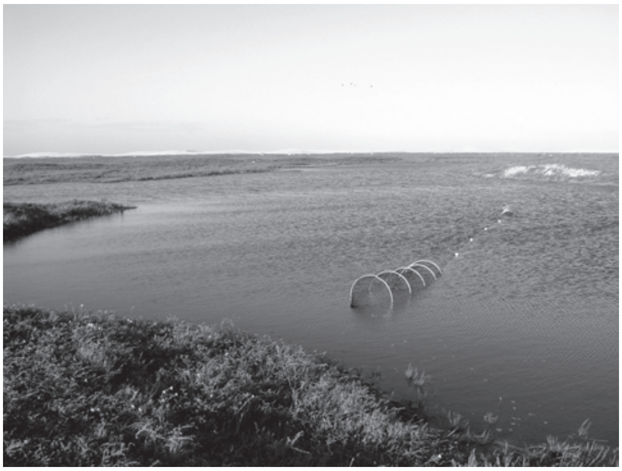

FIGURE 60 Fyke net deployed in a river. A Fyke net consists of two single-mouth hoop traps set on either end of a lead. The traps may be baited to attract certain species of turtles. Other species that are not attracted to bait follow the lead into the traps. (Photo courtesy of Alexandre M. Batistella.)

Fyke nets (Fig.60) represent another modification of hoop nets. In Fyke nets, a rectangular net box is attached to the front of each hoop net. A lead connects the centers of the rectangular boxes; the net is most effective if it runs from the surface to the bottom of the water. Wings may also be attached to the sides of each net, extending out from 10 to 20 m, spanning migration routes or paths to and from basking sites and diverting turtles into the traps. This modification increases the efficiency with which herbivorous turtles, which are not attracted to baits, are caught. Fyke nets are the best apparatus for catching a large number and variety of turtles from whatever aquatic habitat; all species, regardless of feeding preference, are caught in these traps. The traps are best to use for long-term studies, but they are also the most cumbersome and most expensive of all turtle traps. D. W. Tinkle and J. Congdon (pers. comm.) in their long-term turtle studies on the George Reserve (ESGR, University of Michigan) made stationary leads; whenever they were ready to sample, they put the traps in the water and attached the leads. The turtles became accustomed to moving along the leads when the traps were absent, which may have enhanced their capture effort.

All investigators must have at least some basic knowledge of where to place their traps in the habitat. All species of aquatic turtles that I have studied can be trapped effectively with turtle traps with leads in all bodies of water. We sometimes had eight species of turtles in a single trap in our studies on the Pearl River in Mississippi (C. J. McCoy and R. C. Vogt unpubl. data). Traps with smaller-diameter hoops and shorter leads (or only wings) are used in smaller bodies of water. A variety of different habitats needs to be sampled in any heterogeneous body of water in order to capture a representative sample of the turtles residing there. Traps should be set with the leads of the trap parallel to basking logs, between basking logs and deep water. *Trionyx* often bask on sand bars, and many species of turtles also nest on sand bars, so nets set parallel to sandbars or in front of nesting areas in 1 to 2 m of water are often effective. *Graptemys* can be trapped in the fast-moving portions of rivers. The nets must be set parallel to the shore line and attached to it in water 1 to 2 m deep. Traps must often be tied in several places to keep at least part of the trap out of the water and also to keep the current from rotating the traps and disabling the leads. Turtles usually forage and feed in water 0.5 to 2 m deep and within 5 to 10 m of the shoreline in deep lakes and rivers. Traps should be set parallel to the shoreline, as turtles often move along the shore line in search of prey or forage plants. Cursory observations made before setting traps are important. Distinctive bite marks on the leaves of shoreline vegetation reveal recent feeding areas of *Dermatemys*. These turtles are also fond of grazing on grasses, so setting traps in front of emergent grass banks should also increase trapping success of this species and of *Staurotypus* spp., which go to grass banks to feed on apple snails. Generally, turtles can often be trapped everywhere in shallow lakes (2–3 m deep) with aquatic vegetation throughout. I successfully collected *Pseudemys alabamensis* by stringing together 10 turtle traps with leads to span 300 m across a large bed of submerged aquatic vegetation in Mobile Bay, Alabama.

TRAMMEL NETS

When turtles are inactive or occupy deep water, they cannot be captured with Fyke nets or turtle traps, so *trammel nets* are used (Vogt 1980b). Trammel nets are made of three layers of nylon netting hung from a common float line and lead line (nylon cord with lead weight core). The two outside nets (called walling) are usually made of #9 nylon twine and have a mesh size of 30 to 75 cm, depending on the size of the turtles to be trapped. The finer inside mesh is made of lightweight multifilament gill netting, with 3- to 12-cm mesh. The inside net hangs loosely between the two outside walls and is 30 percent deeper that the outside walls. When the turtle enters the net, it pushes the lightweight netting through a larger opening in the opposite outside wall forming a pocket of netting in which it is held. The trammel net catches a much wider size range of turtles than a standard gill net. These nets are usually used in sections of 100 m and range in depth from 2 to 6 m. Several of these nets can be strung along the shore parallel to a nesting beach, across bays and inlets into lakes, parallel to the shorelines in rivers, below hibernacula, or with great success in water 4 to 6 m deep where herbivorous turtles are foraging. These nets must be checked at least every 4 h to ensure that the turtles do not drown. Trammel nets alone work only if turtles are moving. Turtles can be stimulated to move and be captured in trammel nets by driving them with a carphorn (Vogt 1980b), even when they are in winter hibernation.

A *carphorn* (Fig. 61) is a sheet-metal funnel that was designed by commercial fishermen on the Mississippi River specifically for driving carp and other schooling fish down river channels into gill nets during the colder months of the year. The funnel, constructed of heavy gauge galvanized sheet metal, is 16 cm in diameter and 21 cm long, tapering into a sleeve 4 cm in diameter. A 4-cm diameter, 3-m-long wooden pole is bolted inside the sleeve. The carphorn is plunged rapidly and forcibly into the water, making a loud popping sound, which drives the fish or turtles. If the funnel is not made from heavy-gauge sheet metal, it will collapse when pounded into the water. Any device that makes a lot of disturbance in the water is perhaps effective. Henry Bates (1863) reported Amazonian Indians beating sticks and branches on the surface of the water to drive *Podocnemis* into their nets. Amazonian fishermen today often beat the surface of the

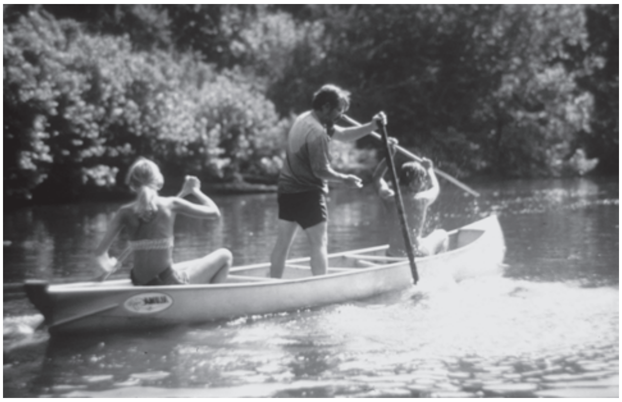

FIGURE 61 Jack McCoy standing in the center of the canoe pounding a carphorn into the water in order to drive turtles into a trammel net. Assisting him are Kelly McCoy (his son) in the bow and Lisa Mattick (RCV's field assistant) in the stern, Glover River, Oklahoma, 1975. (Photo by R. C. Vogt.)

water with canoe paddles to drive fish and turtles out of their hiding places and into their nets.

For target turtles basking on a log, the collector should position the trammel net between the log and the turtles' escape routes as quickly as possible. This is done by driving the boat as fast as is safe to the shore near the basking log; as the boat nears the shore, the driver shifts to reverse. A second person on the bow throws out an anchor attached to a trammel net, feeds the net into the water as the boat makes a semicircle around the basking site, and then throws the anchor attached to the other end of the net into the water. Once the net is in place, the driver moves the boat back and forth between the shore and the basking log, parallel to the net, while the second person plunges the carphorn into the water. I do not know how the sound carries underwater, but turtles move rapidly away in response, entering the net; 50 to 100 turtles can be caught in a matter of minutes. Turtles can be captured this way even when they are hibernating or estivating, as long as the sites where they congregate are known (Vogt 1980b). To be most effective at hibernacula or estivating sites, trammel nets should be weighted so that they sink to the bottom. We have used this technique to capture estivating *Dermatemys* in the deep whirlpools of the Rio Lacantún, in Chiapas, Mexico; for *Podocnemis unifilis* in the Brazilian Amazon; and for the species of hibernating *Graptemys* in the Mississippi River in Wisconsin. Trammel nets are also good for rapidly sampling ponds with diameters of 100 to 300 m. The net is set in the center of the pond, and then the carphorn is pounded into the water from the shore on both sides of the net. Within an hour a large proportion of the turtles will have been captured. This procedure eliminates the wait for the trapping results, as well as loss of nets or traps, and the stomach contents of the turtles are fresh and unadulterated by trap bait.

Data from Terrestrial and Freshwater Turtles

Documenting the presence of turtles and analyzing their community structure or population trends are complex processes; sampling by land, air, and water may be required to amass sufficient data. All sampling methods are probably biased toward a particular sex, size class, or species of turtle. Ream and Ream (1966) discussed the problems with some of the methods. With long-term mark and recapture studies, however, biases can be detected and corrected in the analysis. One problem related to sampling bias is the misconception that the sex ratio in a turtle population should be 1:1 and that any drastic deviation from that ratio indicates a sampling bias. We have documented unequivocally, however, that sex is determined by incubation temperature in many species of turtles (Bull and Vogt 1979), that hatchling sex ratios of some species in any given year can be highly biased in favor of one sex or the other (Vogt and Bull 1984), and that the adult sex ratio of some species of turtles is highly biased in nature no matter how they are collected (4:1 females to male, Vogt 1980a); consequently, some of these techniques may not be biased.

Turtles densities are usually reported as number of turtles caught per trap hour per m of shoreline, or as number of turtles caught per net hour per m of trammel net. Basking turtles are reported as number seen per km of river or shoreline during a set period. Nest counts are reported per hectare of nesting habitat. Dead turtles on road (DTOR) are reported as number per km of road.

Marine Turtles

Documenting the presence of any of the seven species of marine turtles on a nesting beach can be a relatively simple process of knowing when nesting occurs and then walking the beach at night with a flashlight and identifying and counting each turtle encountered. Nests and tracks can also be counted. Because many species of sea turtles lay multiple clutches in a season and individuals of some species do not reproduce every year, one must be careful when speculating about the number of reproductive females of each species that uses a particular nesting beach. It is perhaps safer to calculate the number of nests that are constructed and the number of hatchlings produced to determine the potential importance of a nesting beach for a particular species. With only seven species of marine turtles, investigators can easily learn to identify them and their tracks, nests, and eggs by sight. Investigators with all-terrain vehicles (ATVs) can transverse hundreds of kilometers of beaches each day counting turtle tracks and nests. Many researchers have counted nests and tracks during airplane flights. Bjorndal (1999) reported that even though sea turtles spend at most 1 percent of their lives on or around nesting beaches, about 90 percent of the literature on sea turtle biology is based on studies at nesting beaches. The reasons for this are obvious; in Bjorndal's (1999, p. 12) words, "Anyone who has spent days on rough seas searching for turtles and finding them at a rate of one per day cannot help but think wistfully of the colleague working on the nesting beach who, during a pleasant evening stroll, is certain to encounter many more turtles." As long as granting agencies support additional beach studies in conjunction with the numerous sea turtle conservation programs, rather than insisting that funding be used to support new and innovative research and management during the other 99 percent of the lives of these creatures, populations will continue to dwindle. Information on habitat use of all species and size classes of turtles when they are away from the nesting beaches is desperately needed. Nevertheless, data on the size and condition of nesting habitats, density of nests, survivorship of nests, and nest temperatures are important. With an ever-decreasing amount of money available for conservation and management, it will be impossible to protect all beaches; beaches with the highest survivorships, densities, species diversities, and numbers of female hatchlings should be of highest priority for protection (Vogt 1994).

Acknowledgments

I would like to thank my mentors, the late Jack McCoy and John M. Legler, for the hundreds of hours they spent over the last 40 years suffering and answering my questions. I also recognize Mike Ewert for finally admitting that driving turtles into a trap with a carp horn is a more effective and efficient technique than hand-capturing or noodling. I also thank my many assistants who suffered my wrath and impatience while field testing these methods over the years: Marsha Christiansen, Lisa Mattick, Kris Klevickis, Jan Salick, Mike Pappas, Oscar Flores Villela, Claudia Carnevale, Veronica Gonzales, Marco Antonio Lopez, Nora Tamal Lopez, Paco Soberon, Alan Jaslow, Marcelo Paxtian, Santos Mataperro, Rafael Bernard, Oneide Ferreira da Cruz, Mario Pinto Castro, Augustin El Gato, Virginia Bernard Diniz, Camila Rudge Ferrara, Larrisa Schneider, and Carla Eisenberg.

Alternative Methods for Sampling Freshwater Turtles and Squamates

Thomas S. B. Akre, John D. Willson, and Thomas P. Wilson

Conventional techniques for sampling aquatic turtles and terrestrial squamates are well developed with a proven track record (Lagler 1943; Balgooyen 1977; Plummer 1979; Jones 1986; Karns 1986; Fitch 1987; Dunham et al. 1988; Gibbons 1990; Heyer et al. 1994; Schemnitz 1996). However, no single capture method is effective in all habitats, nor can it be applied to all species and all life stages with equal success (Moll and Legler 1971; Campbell and Christman 1982; Gibbons 1983, 1990). Certain aquatic habitats may be challenging to sample because they are relatively remote and/or because of the nature of the waterscape and its substrate and vegetation (e.g., small fast-flowing rivers, forested swamps, bogs, or ephemeral wetlands). Just as often, aquatic species can be difficult to sample because of aspects of their life history (e.g., diet, microhabitat use, ontogenetic variation, or relative abundance). For example, the turtles *Pseudemys concinna, Clemmys guttata,* and *Graptemys* spp. do not respond well to baited aquatic traps, but all have been readily captured using other techniques (Chaney and Smith 1950; Plummer 1979; Vogt 1980b; Gibbons 1990; Graham 1995). Likewise, snakes that favor aquatic vegetation (e.g., *Regina alleni, Seminatrix pygaea*) can be captured in significantly greater numbers by straining aquatic vegetation than by aquatic funnel trapping or visual surveys (Godley 1980, 1982). Therefore, effective sampling often requires specialized techniques that suit the habitat and reflect the life-history traits and ecology of the target species or assemblage.

Below we describe several methods for sampling turtles, snakes, and lizards in aquatic habitats and comment on the strengths and weaknesses of each technique, special considerations, and particularly pertinent applications. Three factors should be considered regardless of the study design, target taxa, or habitat. First is capture probability, which can be enhanced by advanced familiarity with the study area and consideration of season and weather. Second, close attention should be paid to the methods used by indigenous peoples, which have proven fruitful (e.g., see Lamar and Medem 1982; Luiselli 1998; Gaulke et al. 1999). Third, multiple direct and indirect methods (*sensu* Vogt 1980b) should be used across habitats over a long period to maximize sample size and minimize capture bias and its influence on interpretation of data (Ream and Ream 1966; Wilbur 1967; Moll and Legler 1971; Overton 1971; Vogt 1980b; Gibbons 1983, 1990; Henke 1998). Finally, new techniques for the ancient problem of capturing organisms emerge regularly. We suggest that our list, while not exhaustive, is thorough and therefore includes the basic methods. Nonetheless, an investigator should always consult the recent literature when developing a study design.

Active Capture

VISUAL SURVEYS

Visual surveys are often used for reconnaissance of turtle presence, habitat use, activity, and abundance and can also be used for basking aquatic snakes and certain lizards (e.g., *Varanus* spp., *Tupinambis* spp.). With a well-honed search image, the presence of turtles, snakes, and large lizards can be verified and quantified by scanning the habitat with the unaided eye, binoculars, or spotting telescopes. Scanning from an elevated location or a blind enhances the viewing area without provoking a flight response from basking turtles (Moll and Legler 1971; Holland 1994; Lindemann 1996). Researchers have used visual surveys to locate and count surface basking or breathing *Apalone spinifera, Chelydra serpentina, Chrysemys picta,* and *Kinosternon* spp. (Mosiman and Bider 1960; Webb and Legler 1960; Teska 1976; Lovich 1988; Iverson 1989) as well as aerial basking (i.e., from a raised location such as a log or rock) *Graptemys* spp., *Pseudemys concinna, Rafetus euphraticus,* and *Sternotherus carinatus* (Chaney and Smith 1950; Conant et al. 1964; Moll 1986; Lindeman 1996, 1998, 1999; Taskavak and Atatür 1998). Scanning from a boat has been used successfully to locate and capture *Chelydra serpentina, Chrysemys picta,* and *Emydoidea blandingii* and for distribution surveys of *Actinemys marmorata* and *Dermatemys mawii* (Lagler 1943; Mosimann and Bider 1960; Gibbons 1968a; Moll 1986; Holland 1994).

HAND CAPTURE

Hand capture has been used in diverse habitats for sampling many aquatic reptile species (e.g., Mosimann and Bider 1960; Mahmoud 1969; Moll 1976; Mushinsky and Hebrard 1977; Mushinsky et al. 1980; Auffenberg 1981; Hulse 1982; Lamar and Medem 1982; Shively and Jackson 1985; Dundee and Rossman 1989; Gibbons 1990; White and Moll 1992; Greene et al. 1994, 1999; Flores-Villela and Zug 1995; Souza and Abe 1995, 1997; Magnusson, Cardoso de Lima, Lopes da Costa, and Vogt 1997; Bury and Germano 1998; Greshock 1998; Luiselli 1998; Mills 2002). Many species can be captured effectively by hand, dipnet, or pole snare while the investigator walks a shoreline, wades, floats, floats in an inner tube, or rides in a small boat. Nonetheless, success will likely vary by target species and the skill of the investigator (Moll and Legler 1971; Gibbons 1983). Some species may be readily captured when approached, but others may have to be ambushed (e.g., *Apalone mutica, Phrynops*

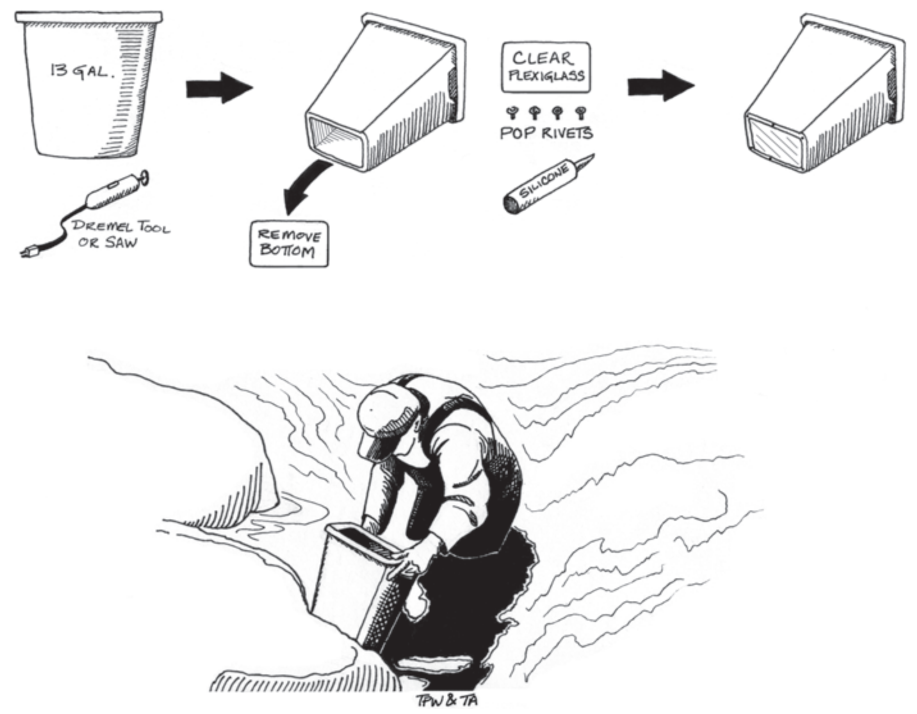

FIGURE 62 Trash-can-model aquatic viewscope used to locate and observe turtles that are underwater.

williamsi, and *Trachemys stejnegeri*; Hodson and Pearson 1943; Fitch and Plummer 1975; Ward et al. 1976; Buskirk 1989). When the element of surprise is necessary to capture reptiles as they bask over water, the researcher can float with his or her body concealed below the water's surface or use a floating blind (Bider and Hoek 1971; Shealy 1976; Gibbons 1983; Mills 2002). One drawback to hand capture is that only conspicuous, active animals are typically captured, which may result age and/or sex-biased data (Dunham et al. 1988).

VIEWSCOPES

Aquatic viewscopes have long been used in aquatic ecology and fisheries research (M. J. Pinder, pers. comm.) and can be adapted to aid in turtle capture in clear shallow ponds, streams, and rivers. Although aquascope designs vary, they all function by breaking the water's surface and reducing surface distortion and interference from reflected light. An aquascope can provide an alternative to diving if the researcher is outfitted with chest waders and the substrate can be reached by hand or dipnet. A small plastic trash can with a silicone-sealed clear, Plexiglas bottom (Fig. 62) is invaluable for observing and capturing juvenile and adult *Glyptemys insculpta* in the cooler months (Akre 2002). Likewise, fiber-optic scopes can be used successfully to locate *Gopherus agassizii* in burrows, *Mauremys japonica* under water, and *Sistrurus catenatus* in crayfish burrows and other underground refugia (Cairns 1983; Yabe 1992; Purcell 1997; Mauger and Wilson 1999; Wilson et al. 1999). Both technologies are particularly useful for locating animals that would otherwise be missed, such as those under cutbanks and submerged structures where vision is normally obscured.

NIGHT SPOTTING

Spotlighting at night with a high-powered lamp has led to the capture of many aquatic reptiles, including both diurnal and nocturnal species. Lagler (1943) first reported success in capturing *Sternotherus odoratus* from a boat using a dipnet and a jacklight. Ernst, Cox, and Marion (1983, 1989) found that wading at night with a lantern was second only to trapping for collecting *S. depressus*. Gibbons (1990) located *Trachemys scripta* with a flashlight in shallow portions of some ponds as they lay quiescent on the bottom during the winter. Moll (1986) used a motorboat and a spotlight to survey *Dermatemys mawii* as they fed and loafed at the surface in lagoons and rivers in Belize. Chaney and Smith (1950) captured hundreds of riverine turtles (e.g., *Apalone spinifera, Chelydra serpentina, Graptemys* spp., *Macrochelys temminckii, Pseudemys concinna, Sternotherus carinatus,* and *Trachemys scripta*) by using a motorboat at night to visit areas of known daytime basking aggregations and then spotlighting and dipnetting the turtles.

Many aquatic snake species are primarily nocturnal, particularly during hot weather. In some instances, these species can be captured effectively at night by carefully searching aquatic habitats with the aid of a flashlight or spotlight. Neill (1964) used this technique to capture extraordinary numbers of rainbow snakes (*Farancia erytrogramma*) in the southeastern United States. Likewise, nocturnal surveys of *Nerodia* spp. and *Regina grahamii* were substantially more effective than diurnal surveys during the hot summer months in Louisiana (Mushinsky and Hebrard 1977; and Mushinsky et al. (1980). Additionally, Corben and Fellers (2001) described a technique for viewing the eyeshine of small reptiles and amphibians that would doubtless prove useful in aquatic surveys of squamates.

DIVING

Snorkeling and scuba diving have proven invaluable for sampling turtles in clear water. Carr and Marchand (1942) invented a technique called *water goggling* in which a diver in the water alongside and propelled by a motorboat uses a facemask to scan for turtles (Marchand 1945). When a turtle is spotted, the diver pushes off the boat and swims after it. This technique allows the investigator to cover more area than with snorkeling alone. Nonetheless, with fins, a facemask, and a snorkel tube, divers in any clear, open aquatic habitat can capture many species of turtle by hand (Gibbons 1968a, 1968b, 1983; Jackson 1969; Moll and Legler 1971; Shealy 1976; Iverson 1977, 1979b; Legler 1978; Legler and Cann 1980; Shively and Jackson 1985; Kramer 1986; Moll 1986, 1990, 1994; Buskirk 1989; White and Moll 1992; Kuchling and Mittermeier 1993; Holland 1994; Magnusson, Cardoso de Lima, Lopes da Costa, and Vogt 1997; Allanson and Georges 1999). Scuba has been employed less frequently, but Graham and Graham (1992, 1997) and Ultsch et al. (2000) used it year round to observe and capture *Apalone spinifera* and *Graptemys geographica* in a Vermont river. Both of these methods greatly enhance sampling success by providing the researcher direct access to turtles in their aquatic habitats.

TRACKING

When turtles are not readily visible, their presence can still be recorded based on sign they leave. For example, individuals of *Apalone mutica* and *A. spinifera* in transit across beaches leave foot and tail tracks, whereas buried individuals leave circles of disturbed sand (Lyons 1972; Williams 1975; Plummer 1977a). Visual tracking of this type has also been used to locate and capture *Kachuga tentoria, Amyda cartilaginea, Cyclemys dentata,* and *Heosemys grandis* in streams and rivers (Singh 1985; Thirakhupt and van Dijk 1994).

Scent-tracking dogs have long been used in wildlife studies and by local hunters for the Asian turtle trade (Zwickel 1980; Hendrie 2000; Platt et al. 2000). Their potential value for turtle research was first noted by Carr (1952), and since then, a few investigators have used dogs to locate turtles (e.g., *Terrapene* and *Kinosternon* spp.; Schwartz and Schwartz 1974; Morales-Verdeja and Vogt 1997).

BLIND CAPTURE

Nonvisual capture takes many forms and may be tailored to the specific study design. Researchers can "muddle" or "noodle" for their quarry by blindly probing the substrate with their hands and feet, or a pole, to feel for the presence of certain turtles and large aquatic snakes (Lagler 1943). This technique has been used in several different species in many different habitats (e.g., *Apalone spinifera, Chelodina* spp., *Chelydra serpentina, Clemmys guttata, Cyclemys dentata, Glyptemys* spp., *Kinosternon* spp., *Lissemys punctata, Terrapene coahuila,* and *Trachemys* spp. (Bishop and Schoonmacher 1921; Hodson and Pearson 1943; Mosimann and Bider 1960; Webb et al. 1963; Lyons 1972; Rhodin and Mittermeier 1976; Auffenberg 1981; Ernst 1976; Ernst, Zappalorti, and Lovich 1989; Gibbons 1990; Moll 1990; Thirakhupt and van Dijk 1994; Lewis and Ritzenthaler 1997; Lewis and Faulhaber 1999). Although muddling had been most widely used for capturing turtles, Rivas (2000) successfully located green anacondas (*Eunectes murinus*) by prodding shallow hyacinth beds with a pole or his feet in the llanos of Venezuela. In each case, the researcher learned to feel or hear a cue indicating the location of an animal. For additional information, see "Hand Capture" in the section on "Detecting and Capturing Turtles in Freshwater Habitats," above.

STRAINING AQUATIC VEGETATION

Shallow aquatic habitats with abundant vegetation can support large numbers of reptiles but are difficult to sample using conventional methods (Goin 1943; Godley 1982). Often, animals can be located by raking the substrate or vegetation with a potato or garden rake (Goin 1942; Plummer 1977b; Ernst, Cox, and Marion 1989). In addition, Godley (1982) modified a sieve originally described by Goin (1942) and used it to sample aquatic snakes in beds of Florida water hyacinth (*Eichhornia crassipes*). Large numbers of *Regina alleni, Seminatrix pygaea, Farancia abacura, Nerodia floridana,* and *N. fasciata* were captured with the sieve, which consisted of a 100-×50-×10-cm wood frame with a bottom of plastic window screen supported beneath by 0.5-in hardware cloth (Godley 1980, 1982). These sieves can also be used to sample small turtles, semi-aquatic lizards, and other aquatic reptiles. Additionally, it is more quantitative than other methods, as it allows for complete sampling of a known area of habitat and can be used to calculate reptile density and biomass (Godley 1980, 1982).

ELECTROSHOCKING

Electroshocking is a method common to fisheries research (Murphy and Willis 1996) that may be useful for sampling reptiles in clear shallow water from which stunned animals can easily be retrieved (Gunning and Lewis 1957; Harris 1965; Dobie 1971). Although published accounts of electroshocking reptiles are few, T. Mills (pers. comm.) successfully captured *Nerodia floridana* and *N. taxispilota* in swamps and streams of South Carolina using this technique. In addition, ichthyologists have reported capturing *Glyptemys insculpta* and *Farancia erytrogramma* (a species notoriously difficult to find) while electroshocking eels in streams and small rivers (D. Fletcher, pers. comm.; F. Frenzel, pers. comm.).

Trapping

AQUATIC FUNNEL TRAPS

Aquatic funnel traps are among the most effective means of sampling inconspicuous aquatic squamates and have been used as the primary sampling technique in numerous studies of aquatic snakes (e.g., Greene et al. 1994, 1999; Seigel, Gibbons, and Lynch 1995; Seigel, Loraine, and Gibbons 1995). Although numerous funnel-trap variations have been described, commercially available minnow, crawfish, or eel traps remain the standard for aquatic situations (Adams et al. 1997). These are generally constructed of metal hardware cloth, tough plastic, nylon mesh, or wood (Adams et al. 1997). Traps are generally set in shallow water, allowing captured animals access to air (Adams et al. 1997). However, Casazza et al. (2000) attached polystyrene floats alongside eel pots to allow for surface trapping of

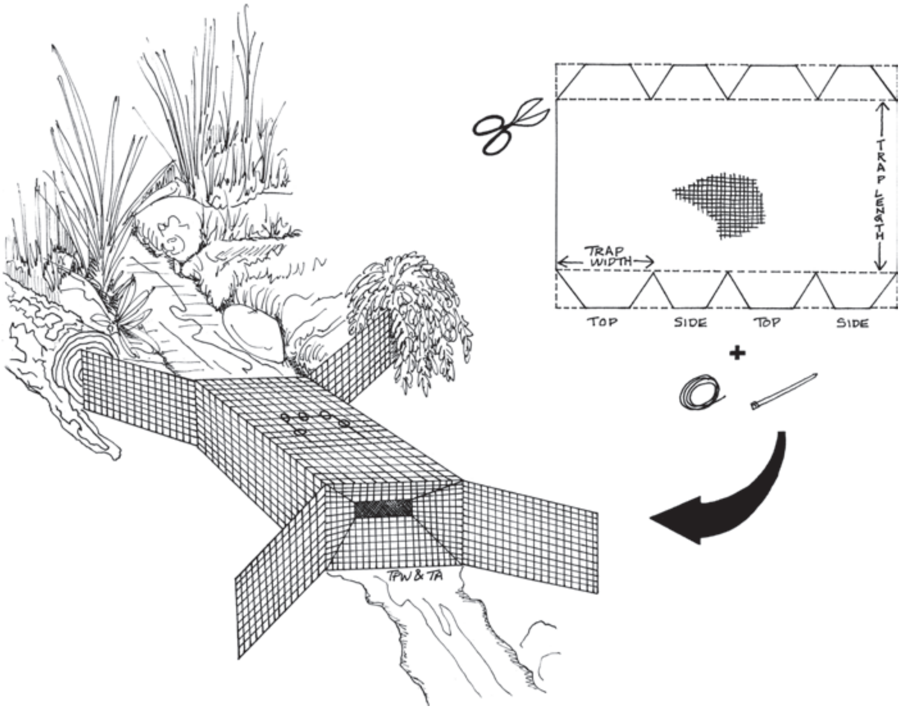

FIGURE 63 Swing-door box trap for catching turtles. The hanging door opens in only one direction, so that once a turtle enters the trap, it is unable to exit.

Thamnophis gigas in deep-water habitats. Although funnel traps will often "bait themselves" with bycatch, Keck (1994a) found that traps purposefully baited with dead sunfish (*Lepomis* sp.) and tadpoles (*Lithobates catesbeianus*) captured more than twice the number of semi-aquatic snakes than did unbaited traps in Texas ponds.

INTERRUPTION TRAPS

Small- to medium-size traps constructed of poultry netting or hardware cloth, when placed in a naturally occurring bottleneck, interrupt the movement of turtles and squamates and trap the animals without bait. These interruption traps can be constructed to fit any size passage in any microhabitat, aquatic or terrestrial. Funnel traps, which have been used in numerous turtle studies, have an opening at each end to lead turtles into a holding chamber (Legler 1960a; Gibbons 1968b; Iverson 1979a). Swing-door box traps have a hanging door that opens in only one direction so that once a turtle has entered it is unable to exit. Both trap designs may be complemented with wings (i.e., drift fences) constructed of wire or fencing that lead off each side at a 45-degree angle (Fig. 63). Interruption traps have been used to capture *Clemmys* and *Glyptemys* spp., among many other species (Ernst 1976; Wilson 1994; Akre 2002).

LURES

Researchers have long suggested the value of placing female turtles into hoop nets (see "Baited Hoop Traps" in the section on "Detecting and Capturing Turtles in Freshwater Habitats," above) to lure conspecifics into the trap (Cagle and Chaney 1950; Plummer 1979; Gibbons 1983; Dunham et al. 1988). Frazer et al. (1990) evaluated this technique and concluded that turtles are attracted to traps containing conspecifics. Likewise, Mansfield et al. (1998) placed hand-painted decoys in traps and successfully attracted *Clemmys guttata*. This technique evidently can also be used to sample aquatic squamates and the addition of live animals or decoys to traps should be considered whenever practical.

AQUATIC DRIFT FENCES

Traps placed along natural "drift fences" such as submerged logs, steep shorelines, and swamp channels capitalize on the natural thigmotaxis by capturing animals that are moving along these barriers (Fitch 1987; Keck 1994a). Enge (1997a) provides detailed descriptions of aquatic drift fence construction using silt fencing and wire funnel traps, but the efficacy of drift fences in comparison with traditional funnel traps for capturing aquatic reptiles is poorly established (Willson and Dorcas 2004b). Willson and Dorcas (2004b) described options of a similar design, using collapsible nylon mesh minnow traps along short (3-m) sections of silt fencing. This design of drift fence captured nearly three times the number of amphibians than unfenced traps did in a temporary wetland in North Carolina. Although further research is necessary, it is likely that aquatic drift fences would prove similarly effective for capturing aquatic squamates and small turtles. Important considerations in construction of aquatic drift fences are that trap openings lie flush against the fencing and that some part of the trap remains above water to ensure that trapped animals have access to air (Enge 1997a; Willson and Dorcas 2004b).

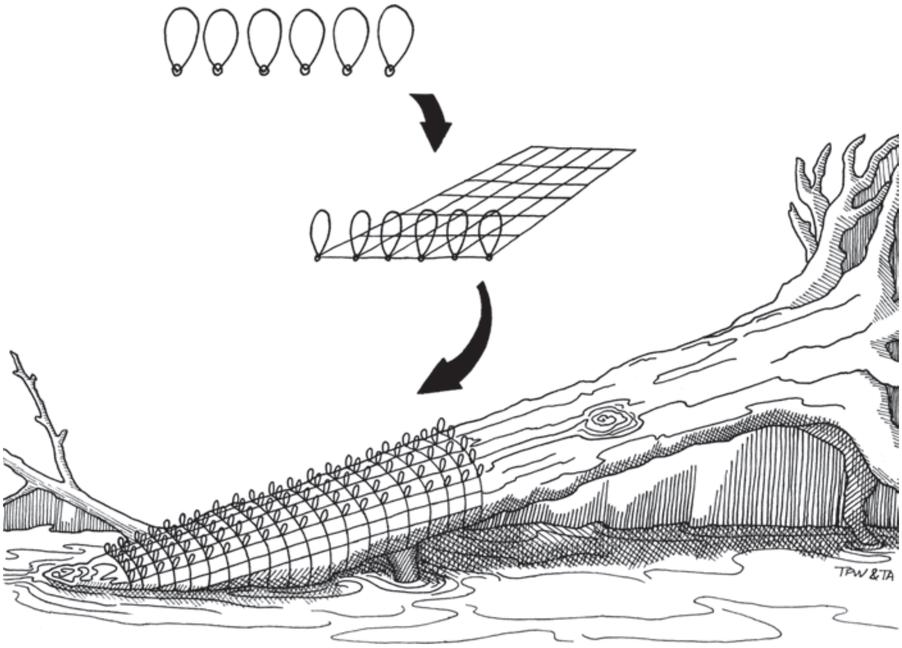

FIGURE 64 Bal-chatri trap made from a piece of wire mesh (e.g., hardware cloth, chicken wire) to which a series of erect slip nooses made of monofilament line are attached. When turtles (or other animals) cross the wire, the loops act as snares.

MIST NETTING

Mist netting, a trapping method commonly used in ornithology (Bub 1991) and not unlike gillnetting for fish and turtles, has been used to capture aquatic snakes (*Nerodia rhombifer, N. erythrogaster,* and *Agkistrodon piscivorus*) in Oklahoma streams and ponds (Lutterschmidt and Schaefer 1996). However, the method may be biased toward the capture of large snakes, and removal of venomous species from the net may be problematic (see "Handling Live Reptiles," in Chapter 8).

SNARES

Traps employing a snare mechanism are commonly used in studies of mammals and birds. The bal-chatri trap, invented by Berger and Mueller (1959) consists of a wire mesh platform and a series of erect loops made of a monofilament line that act as snares (Fig. 64). Braid (1974) suggested its use on/around basking sites or in areas of high use, and found it effective in the collection of *Chrysemys picta* and *Clemmys guttata*. Shively and Jackson (1985) used it as their principal method for capturing *Graptemys* spp. Reed et al. (2000) found a baited version of this design to be the most effective method for the capture of *Varanus indicus* in the Mariana Islands. Although effective, bal-chatri traps may harm incidentally captured wildlife and should be checked frequently to reduce the chance of injury and mortality (Vogt 1980b; Reed et al. 2000).

Varanid lizards have been captured effectively in terrestrial and arboreal habitats using a variety of methods, including burrows excavation, trapping, noosing, pit trapping, and snaring (Auliya and Erdelen 1999). Of these techniques, only snares baited with meat or fish proved useful for capture of large aquatic monitors (e.g., *Varanus salvator*; Shine et al. 1996), and Gaulke et al. (1999) found that lengthening the string or using a smaller angle of stick greatly reduced incidence of injury. In contrast, Auliya and Erdelen (1999) concluded that the bamboo box trap (similar to a conventional live trap with a trapdoor and solid floor to prevent lizards from digging out) baited with meat or fish was the most effective trap for *V. salvator.* They further improved this design by placing the trap on a bamboo raft to allow trapping of flooded areas and prevent loss of traps in floods.

Application of Terrestrial Techniques for Aquatic Species

Despite their "aquatic" designation, many freshwater reptile species are found regularly in terrestrial habitats, especially those adjacent to aquatic habitats, particularly when dispersing or moving between ponds (e.g., Fitch 1949; Hellman and Telford 1956; Campbell and Christman 1982; Dalrymple et al. 1991; Bernardino and Dalrymple 1992; Tucker 1995). Consequently, they are often registered or captured with methods normally deployed for surveying terrestrial species. These techniques are discussed at length in other sections (e.g., see above and Chapters 5 and 13) and so will be mentioned here only briefly.

Road riding (see "Road Riding," in Chapter 13) can be particularly useful for species inventories and often allows many species to be recorded in a short time. In addition, road kills can provide specimens. Artificial cover objects (see "Sampling with Artificial Cover," in Chapter 13) is another terrestrial technique that can be applied to sample aquatic species, particularly if the cover objects are near to or even partially submerged in water (Grant et al. 1992; Seigel, Loraine, and Gibbons 1995). Likewise, terrestrial drift fences and traps (see "Funnel Traps, Pitfall Traps, and Drift Fences," in Chapter 5, and "Pitfall-Trap Surveys," in Chapter 13) located near aquatic habitats will generally capture the most aquatic species. In fact, terrestrial drift fences have served as the primary sampling method for studies of many aquatic turtle and snake species (e.g., Gibbons et al. 1977; Semlitsch et al. 1988; Dodd 1992a, 1993a; Seigel, Gibbons, and Lynch 1995; Seigel, Loraine, and Gibbons 1995).

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Sampling Marine and Estuarial Reptiles

Harold K. Voris and John C. Murphy

When one thinks of environments occupied by reptiles, saltwater and brackish-water habitats are not the first that come to mind. However, a variety of species routinely occupy marine habitats, and others are occasional visitors. Schmidt (1951) and Neill (1958) compiled global surveys of reptile species known to use brackish and saltwater environments, as well as species known to occur in habitats adjacent to the sea and estuaries. In this section, we focus on reptile species that occupy marine habitats primarily, including salt marshes, estuaries, mangroves, and other coastal environments.

Among modern reptiles, snakes are by far the most successful in marine habitats, although the Galapagos Marine Iguana (*Amblyrhynchus cristatus*), one crocodilian, the Salt Water Crocodile (*Crocodylus porosus*), and seven species of sea turtles (families Cheloniidae and Dermochelyidae) also make use of this habitat. One species of file snake (*Acrochordus granulatus,* Acrochordidae) is found almost exclusively in brackish water and marine habitats. The other two species, *A. javanicus* and *A. arafurae,* are primarily freshwater species, but they too have been reported from brackish water and marine environments. Most of the 37 species of homalopsid snakes (Homalopsidae) are freshwater, semi-aquatic, and aquatic species, but at least 10 species spend part or much of their lives in brackish or salt water (Murphy 2007). See Pauwels et al. (2008) for a summary of global diversity of freshwater snakes.

Sea snakes (Subfamily Hydrophiinae, Family Elapidae) are usually marine, but a few species have returned to freshwater. The Hydrophiinae are the most diverse group of marine snakes today and represent a relatively recent radiation (Lukoschek and Keogh 2006). Culotta and Pickwell (1993) have compiled an extensive bibliography on the Hydrophiinae.

Sea kraits of the genus *Laticauda* are also included in the family Elapidae but represent an independent lineage from the Hydrophiinae that some recognize as a separate Subfamily Laticaudinae. The Sea kraits are semi-aquatic in island environments, and while well adapted for marine life with a paddle tail, they bask, mate, digest food, and lay eggs on land. Keogh (1998), Dunson (1975a), Heatwole (1999), Heatwole, et al. (2005), and Ineich and LaBoute (2002) have discussed their biology.

Other lineages of lizards and snakes have scattered species or populations that have specialized in coastal habitats. Although many of these species are terrestrial or arboreal, they also use marine resources. For example, arboreal Burmese Vine Snakes (*Ahaetulla fronticincta,* Colubridae) ambush marine mudskippers (fish in the family Gobiidae, subfamily Oxudercinae) from arboreal perches overhanging the water (Smith 1943).

Three North American natricines, the Saltmarsh Watersnake (*Nerodia clarkii* ssp.), the Marsh Brownsnake (*Storeria dekayi limnetes*), and the Cape Gartersnake (*Thamnophis valida*), are coastal snakes that use mangroves and salt marsh habitats (Conant 1969; Gibbons and Dorcas 2004; Anderson 1961; Rossman et al. 1996). Additionally, some North America populations of the semi-aquatic pit viper, the Florida Cottonmouth (*Agkistrodon piscivorous conanti*), use mangroves and salt marshes (Lillywhite et al. 2008). In the Neotropics, some species of the aquatic and semi-aquatic dipsadid genera *Helicops, Hydrops, Liophis,* and *Tretanorhinus* use freshwater and brackish water in coastal habitats to varying degrees.

In North Africa and Europe, three natricid species use coastal habitats: the Viperine Snake, *Natrix maura*; the Grass Snake, *N. natrix*; and the Dice Snake, *N. tessellata* (Schleich et al. 1996; Sindaco et al. 2006). Two African snakes (*Grayia smythii* and *Crotaphopeltis hotamboeia*) of uncertain lineages occur in brackish-water, mangrove habitats as well as in freshwater (Luiselli et al. 2002; Kelly et al. 2009).

In Southeast Asia, the Mangrove Pitviper (*Cryptelytrops purpureomaculatus*), the Mangrove Snake (*Boiga dendrophilia*), and the Hamadryad (*Ophiophagus hannah*) inhabit mangroves, but like the Vine Snake, these species are mostly arboreal and terrestrial, and therefore, unlikely to spend significant time in the water.

Habitats

ESTUARIES AND RIVER MOUTHS

All three species of file snakes (*Acrochordus* spp.) have been reported from these habitats. Many species of Hydrophiinae use estuaries and river mouths, particularly *Aipysurus eydouxii, Enhydrina, Hydrelaps, Lapemis, Parahydrophis,* and some *Hydrophis* (e.g., *brookii, lapemoides, melanosoma, macdowelli, obscurus, ornatus, torquatus,* and *vorisi*). It is also likely that the 10 species of homalopsids known to use brackish and salt waters use these environments at least on occasion. Many homalopsids that are primarily freshwater species may live in river mouths. For example, at least six species of the mostly freshwater *Enhydris* have populations in river mouths, and three of these species (*E. maculosa, E. pakistanica, E. vorisi*), appear restricted to river deltas. A few of the natricids and dipsadids previously discussed also use these habitats.

MANGROVE SWAMPS

North American aquatic snakes that use mangrove habitats include *Nerodia clarkii* ssp. and some populations of *Agkistrodon piscivorous conanti*. Wharton (1969) and Lillywhite et al. (2008) reported *A. p. conanti* scavenging for food under nesting herons and cormorants as well as in the intertidal zone. In the Neotropics *Helicops, Hydrops, Liophis,* and *Tretanorhinus* may be present in mangroves at least occasionally. In Australasia, mangroves are used by homalopsids, particularly *Cerberus, Cantoria annulata, Fordonia,* and *Myron*. In Southeast Asia *Bitia, Cerberus, Cantoria violacea, Fordonia,* and *Gerarda* use these habitats.

SALT MARSHES

Few snakes are specialized for inhabiting salt marshes; even those that use mangroves, estuaries, and river deltas are unlikely to occur there. North America's *Nerodia clarkii* and *Storeria dekayi limnetes* are known to use salt marshes. Bennett's Mud Snake (*Enhydris bennetti*), a homalopsid, is frequently reported from the salt marshes and mangroves of coastal China, from Hong Kong to Hainan and North Vietnam (Murphy 2007; Nguyen et al. 2009). Based on current knowledge, this species appears to be an intertidal specialist (Murphy 2007). In Scilly, Luiselli et al. (2005) found *Natrix n. sicula* feeding on marine fish in a highly degraded coastal marsh. The terrestrial snakes *Coluber viridiflavus* and *Elaphe lineata,* as well as several species of terrestrial lizard, were also collected at that study site.

Sea-grass beds may occur in relatively clear water, facilitating visual inspection of snakes. Kerford, et al. (2008) were able to count Bar-bellied Sea Snakes (*Hydrophis elegans*) from a boat traveling about 7 km per hour in water that was approximately 3.5 m deep at Shark Bay, Western Australia.

Capture

Marine and estuarial environments present a huge array of structural diversity. Thus, the best technique to use in one habitat may not function in another habitat. In addition, the daily tidal fluctuations, as well as variation in substrate type (ranging from rock and sand to mud) and vegetation density (from algal mats to dense Nipa Palm [*Nypa fruticans*] or mangrove), make the intertidal habitat particularly challenging to researchers wishing to sample the herpetofauna. We have successfully used funnel traps (see "Funnel Traps, Pitfall Traps, and Drift Fences," under "Trapping," in Chapter 5) to sample freshwater populations of homalopsids in Thailand, but these traps are not practical in tidal environments, because of the tidal fluctuations and water movement. A summary of sampling techniques follows.

OTTER TRAWLS

Otter trawling, or *dragging*, which is commonly used in commercial fisheries, involves dragging a large net behind a boat. The net may be dragged along the bottom or in the water column behind the vessel and is attached to the vessel by ropes or steel cable. The net is held open by two large "doors," which are attached to either side of the net. Periodically, the net is hauled aboard the vessel, and the catch is spilled from the bag before the net is redeployed. Mouths-of-Otter trawls range from 5 to 20 m or more across. Sea snakes are frequently part of the bycatch. Through contracts with fishermen, an investigator can obtain large numbers of sea snakes, but details on where the snakes were collected, the nature of substrate, and the depth of the water are nearly always lacking. This problem is compounded because today's modern trawlers routinely travel many hundreds of kilometers over 1 or 2 weeks in search of fish or prawns. In addition, the trawlers often deploy their nets while moving from one fishing ground to another. Thus, although trawlers may provide a large number of specimens, ecological and geographic data are usually missing (Fig. 65). Extensive collections of marine snakes have been made in Tonking Bay (Shuntov 1962) in the South China Sea (Smith 1926; Shuntov 1966; Lemen and Voris 1981), on the Sahul Shelf (Shuntov 1971), in the Gulf of Carpentaria (Wassenberg et al. 1994; Fry et al. 2001), in the Gulf of Thailand (Tu 1974), and off the coast of Borneo (Voris 1964; Stuebing and Voris 1990; Han et al. 1991).

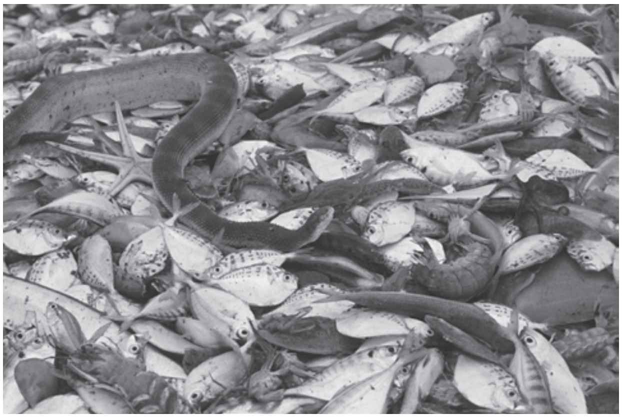

FIGURE 65 Catch from a small commercial otter trawl operating ca. 20 km northeast of Endau, Malaysia, in the South China Sea, 1971. The bycatch is a sea snake, *Thalassophis anomalus*. (Photo by H. K. Voris.)

FIXED STAKE NETS, KELONGS, AND FYKE NETS

In the past, these nets were deployed in river mouths and in shallow coastal areas throughout Southeast Asia and northern Australia. They are all stationary, relying on river flow or tidal currents to carry fish and prawns into the nets. Snakes are again a bycatch in these nets. *Stake nets* consist of a net stretched on stakes fixed into the substrate; the fish become trapped in enclosures at one end of the net and are periodically removed by the fishermen. Lights are often deployed around these structures at night to improve the catch.

A *kelong* (Fig. 66) is an offshore platform built for the purpose of fishing. The fishermen deploy nets by wading or swimming in shallow waters or from boats in deeper water. Although marine snakes are an incidental catch at kelongs, extensive samples have been obtained in Malaysia (Voris and Moffet 1981; Voris 1985) and in tropical Australia (Houston and Shine 1994a).

Fyke nets are bag-shaped nets that are held open by hoops and often outfitted with wings and leaders (Fig. 60; these nets described in detail in "Baited Hoop Traps," under "Detecting and Capturing Turtles in Freshwater Habitats," above). They can be linked together in a series and are used to catch fish moving against the current, but they can also be used in lentic waters with dense submergent vegetation. Fyke nets are used in marine and freshwater situations and are sometimes baited. They are often designed for specific species of fish; however, Houston and Shine (1994a) used them successfully to collect *Acrochordus arafurae* in shallow water.

TARGETED HAND COLLECTING

Targeted marine snakes can be hand collected by dipnetting at night with light from ships (Smith 1926; Dunson and Minton 1978). Dipnetting has also been used to capture Yellow-bellied Seasnakes (*Pelamis platurus*) during the day off the west coasts of

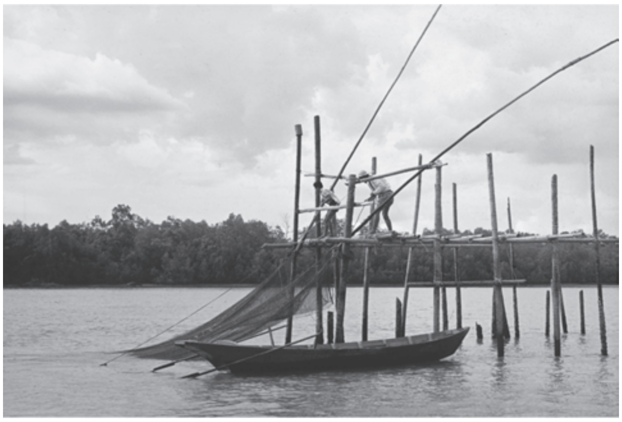

FIGURE 66 Kelong, or stake net, near the mouth of the Muar River, Malaysia, 1975. The net was set at high tide and operated on the outgoing tidal river flow. Prawns were the intended catch, but sea snakes such as *Enhydrina schistosa* and *Hydrophis melanosoma* were common in the bycatch. (Photo by H. K. Voris.)

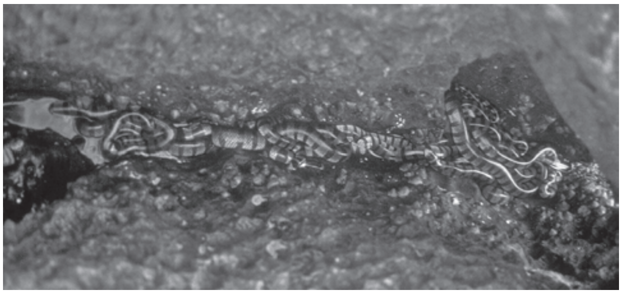

FIGURE 67 A cluster of about 12 Banded Sea Krait (*Laticauda colubrina*) in a rock crevice in the intertidal zone of a small island south of Labuan, Sabah, Malaysia (Borneo). (Photo courtesy of R. B. Stuebing.)

Costa Rica and Panama (Dunson and Ehlert 1971; Kropach 1971) and off the coast of Queensland, Australia (Dunson 1975b).

Sampling and counting marine snakes in clear water while skin and scuba diving has been used effectively in the Philippines (Gorman 1985; Dunson and Minton1978), on the Ashmore (Guinea 1996) and Great Barrier (Heatwole 1975; Burns and Heatwole 1998; Lukoschek et al. 2007) reefs in Australia, and in Taiwan (Tu and Su 1991). In addition, *Acrochordus granulatus* has been sampled by hand by wading in shallow mangrove areas in the Philippines (Gorman 1985; Dunson and Minton 1978).

In general, trawls, stake nets, fyke nets, and other commercial fishing methods are not effective for sampling the amphibious sea kraits (*Laticauda*). Instead, hand collecting in the intertidal and backshore (generally within 50 m of the mean high-tide mark) zones has proven effective for these taxa. Sea kraits are often found on islands (Fig. 67), but they are not restricted to them. Studies have been conducted on *Laticauda* in Taiwan (Tu et al. 1990); the Solomon Islands (McCoy 1985), including Rennell Island (Cogger et al. 1987); Vanuatu (Shine, Reed, Shetty, and Cogger 2002; Shine, Reed, Shetty, LeMaster, and Mason 2002), New Caledonia (Saint Girons 1964), Fiji (Guinea 1994; Shetty and Shine 2002), Andaman Islands (Shetty and Sivasundar 1998), and Malaysian Borneo (Lading et al. 1991; Voris and Voris 1995). In the Philippines, *Laticauda* is hand collected for the exotic leather industry (Punay 1975).

The Oriental-Australasian rear-fanged water snakes in the family Homalopsidae encompass about 37 species in 10 genera (Gyi 1970; Murphy 2007). Ten of these species (*Bitia hydroides, Cantoria annulata, C. violacea, Cerberus australis, Ce. rynchops, Ce.* sp., *Enhydris bennetti, Fordonia leucobalia, Gerarda prevostiana,* and *Myron richardsonii*) inhabit estuaries and river mouths. Additionally, the mostly freshwater *Homalopsis buccata* can be found in river mouths and estuaries. Although their freshwater relatives have been successfully sampled using funnel traps in Borneo (Voris and Karns 1996) and Thailand (Murphy et al. 1999), the estuarial species have not. Trapping aquatic snakes in intertidal estuarine environments is difficult at best because of the daily tidal exchange and soft substrates. Hand collecting in mangroves, intertidal creeks, and mud flats at low tide or on an incoming tide has proven effective in Peninsular Malaysia (Jayne et al. 1995), Thailand (Voris and Jeffries 1995), New Guinea (see catalogue entries for *Fordonia* from the Museum of Comparative Zoology, Harvard University), and Australia (M. Guinea, pers. comm.). The particular characteristics of the intertidal habitats at a given location determine the practicality of sampling. Although many areas maintain snake populations, few are workable. The two primary factors that determine the workability of a site are physical access and the consistency of the substrate. Handmade mud skis (Fig. 68) are ski-like structures made of wood or other materials that can be used to access areas where walking is impossible, but they too have limitations (Jayne et al. 1995). Their use over changing substrates can be tricky, and becoming stuck in the mud is a very real hazard. In the Pak Phang Peninsula of Thailand, for example, fishermen collected the Southeast Asian Bockadam (*Cerberus rynchops*) on soft mud by using a small board to distribute their weight, which allowed them to scoot along on the surface of very soft mudflats and collect snakes, crabs, and mollusks. In addition, the presence of mature saltwater crocodiles precludes fieldwork in some areas!

Luiselli and Akani (2002) studied an assemblage of snakes inhabiting a mangrove forest in southeastern Nigeria over a period of 4 years. They used hand collecting during random searches, as well as pitfalls, drift fences, cover objects, and basket traps with no-return valves placed with barricades. It is not clear from their description if any of these methods were used in the intertidal zone where traps would be flooded by returning tides. However, of the 19 snake species they collected, only two were aquatic, and they represented only 5 percent of the 160 snakes collected; the other species were arboreal or terrestrial. Thus, it seems unlikely that they were setting traps and drift fences in areas regularly inundated by tides.

In Singapore, Karns et al. (2002) used a boardwalk designed for park visitors as a transect to sample the homalopsid assemblage present in the Pasir Ris Park mangrove forest. They surveyed the area on 12 nights between 7 February and 30 March, with the assistance of from 10 to 14 volunteers. The 200-m boardwalk was divided into 20-m segments and had been previously mapped for microhabitats. This method produced a total of 220 homalopsids representing four species. In addition, two intensive surveys lasting throughout the night were conducted. They demonstrated that the snakes were active throughout the night.

Scorpion Mud Lobster (*Thalassina anomala*) mounds present a unique and difficult microhabitat to sample (Karns et al. 2002; Voris and Murphy 2002). These mounds (Fig. 69) serve as refugia for at least two and possibly four species of homalopsid snakes (*Fordonia leucobalia* and *Gerarda prevosti-*

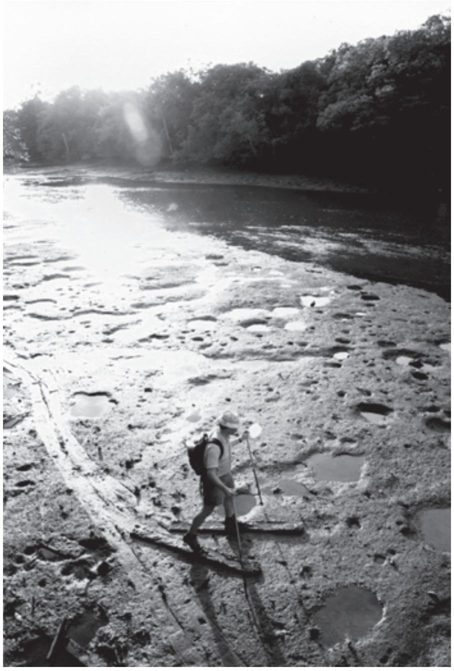

FIGURE 68 Bruce Jayne using mud skis to search for homalopsid snakes (e.g., *Cerberus rynchops*) on the intertidal mud flats of the Johore Straits at Sungei Buloh, Singapore. The consistency of the mud and the mass and physical condition of the skier are critical factors influencing the effectiveness of this mode of collecting. (Photo by Alan Lim. The *Sunday Straits Times* [March 18, 2001, p. 28]; © Singapore Press Holdings Limited; reprinted with permission.)

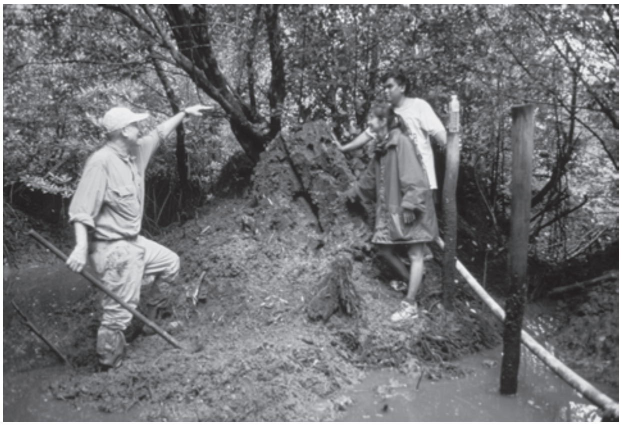

FIGURE 69 Excavation of a large Scorpion Mud Lobster (*Thalassina anomala*) mound in a mangrove forest on the west coast of Thailand. The mounds occur in the upper portion of the intertidal zone and are used by the homalopsid snakes *Cerberus rynchops, Fordonia leucobalia*, and *Gerarda prevostiana*. (Photo by J. C. Murphy.)

ana; possibly *Cantoria violacea,* and *Cerberus rynchops*). The mounds may be 1.5 to 2.0 m high and 10 m in diameter. The only way to remove snakes from them physically is by excavation. Collecting the snakes by hand when they emerge to forage seems a more prudent method of obtaining specimens, to avoid long-term damage to the microhabitat.

The few species of New World snake that inhabit coastal marshes and mangroves have been visually observed and hand collected (Hebrard and Lee 1981; Miller and Mushinsky 1990). Xenodontines (family Dipsadidae) of the genus *Tretanorhinus* occur in freshwater and marine habitats of Central and South America and the West Indies and have been caught in minnow traps and by hand (Neill 1965; Schwartz and Henderson 1991).

Other reptilian taxa that enter coastal habitats include skinks, monitors, iguanids, agamids, and a variety of other snakes. These reptiles have been sampled by hand collecting or studied by simple observation. In Sicily, Luiselli et al. (2005) hand collected squamates in a highly degraded coastal marsh and reported that a mammal research team found several terrestrial skinks (*Chalcides chalcides*) in small mammal traps. The Southeast Asian and Pacific Littoral Skink, *Emoia atrocostata* (Scincidae), inhabits mangrove forests, where it forages in tidal pools on crabs, fish, and insects. Alcala and Brown (1967) conducted a population study on this species in the Philippines and collected samples by hand. Ingram (1979) commented on the littoral and mangrove habitats of this species and observed them being displaced upwards from hiding places in mangrove trees by rising tides. The Coastal Snake-eyed Skink (*Cryptoblepharus litoralis*) has been observed foraging on coral reefs at low tide for marine polychaetes and other littoral invertebrates (Horner 1992); Fricke (1970) observed similar behavior in *Cryptoblepharus boutonii cognatus* in Madagascar. Grismer (1994, 2002) described three species of side-blotched lizards (*Uta encantadae, U. lowei,* and *U. tumidarostra*) from islands in the Islas Las Encantadas Archipelago in the Gulf of California, foraging in the intertidal zone for the marine isopod *Ligia occidentalis*.

Varanus indicus, V. prasinus, and *V. semiremex* inhabit the mangroves of Australia and New Guinea (Green and King 1993). *Varanus salvator* is widespread and common in mangroves and freshwater habitats of Southeast Asia and has been trapped using large live traps (Rashid and Diong 1999; S. M. A. Rashid, pers. comm.). The arboreal pitviper *Cryptelytrops purpureomaculatus* species complex, likely including *Trimeresurus cantori* and *T. labialis* (David and Ineich 1999), appears to be restricted to mangrove forests and to have been sampled only by hand. Techniques used on the Galápagos Islands to sample Galápagos Marine Iguana (*Amblyrhynchus cristatus*) were limited to hand collecting, noosing, and visual observations (Butler et al. 2002; Wikelski and Trillmich 1994; Wikelski and Hau 1995).

The hydrophiids, the homalopsids, a few natricids, and dipsadids are the squamates most likely to be encountered in brackish and salt water. The wide array of structural diversity found in marine and estuarial environments requires sampling techniques to be equally diverse. Further, the daily tidal fluctuations, the softness of the substrate, and the complexity of the vegetation make the intertidal habitat particularly challenging. Large crocodiles make sampling estuarine reptiles in northern Australia hazardous. Marine and estuarine studies in the future could benefit greatly from new innovative techniques.

DATA ANALYSIS AND INTERPRETATION

Reptiles from marine and estuarial habitats are most commonly obtained as bycatch of commercial fishing enterprises and consequently are only rarely accompanied by any data. Consequently, species distribution information (geographic, habitat, depth) is most often second hand at best and of limited use. Exceptions to this include instances where investigators are able to accompany fisherman (e.g. Voris 1985) and where scientific expeditions with research-vessel ship time are involved (e.g., Dunson and Ehlert 1971). Thus, the kinds of analyses that are applicable vary a great deal and must be carefully matched to the circumstances. Even when more detailed data are collected by an investigator, it is very often impossible to determine the area from which the sample is being drawn or whether or not a population is closed. Of course, there are important exceptions to this, such as patch reefs, river mouths, isolated sea mounts, and isolated mangrove patches. In many cases, abundances and densities of species can be calculated when units of effort and area or linear distance can be determined (e.g., snakes per square meter of net per hour of deployment; snakes per meter of boardwalk surveyed; snakes per hour of scuba diving at a particular range of depths). These values can then be used for comparisons through time at a particular place or between locations with mostly shared characteristics.

CHAPTER TWELVE

Statistical Properties of Techniques and Validation

GORDON H. RODDA

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Validation of Techniques and Assumptions / 199

Comparison of Techniques

How does one go about selecting a sampling method? On what basis does one judge a method to be good or bad? Some choices can be eliminated as a simple matter of physical or logistical impracticality. Road cruising will not work for Leatherback Sea Turtles (*Dermochelys coriacea*) or miniscule *Sphaerodactylus* geckos, for example, nor will pitfall traps suffice for those species. Imagine the size of can one would need for a Leatherback pitfall! And geckos with clinging toe pads can climb out of pitfalls. In addition to the requirement that a sampling method be practical, the chosen technique should provide low *bias* and high *comparability, precision,* and *efficiency.*

Bias

Bias is systematic, as opposed to random, error. Random errors scatter measurements symmetrically around the true value. Increased scatter lowers precision (see "Precision," below), but scatter does not distort the average of unbiased measurements. For many monitoring efforts, systematic distortion or bias is the main obstacle to overcome because it sows uncertainty in the interpretation of results. It is often possible to guess the direction of bias, but—by definition—bias has an unknown magnitude (if the size and direction of the bias were known, it would be simple to adjust an estimate accordingly). The amount of bias can also change easily from one measurement to the next, undermining intended comparisons. Suppose for example, that 115 tortoises are estimated to occur in a study area surveyed in 1985, whereas a survey of the same site in1995 yields 138. The investigator suspects that searching was easier or carried out more competently in 1995. What then can the investigator tell the land manager about the trend of the tortoise population? If the bias in 1985 was –18 percent (i.e., the true population was 140) and the bias in 1995 was 0 percent (true population 138), the population technically has declined but for all practical purposes is stable. If instead the 1995 bias is +2 percent, the population has actually increased, although from a manager's perspective it is stable. Other bias scenarios are imaginable for sharply declining, moderately increasing, or sharply increasing populations. In other words, a clear answer is unattainable. Even small amounts of bias can turn an otherwise laudable monitoring program into a waste of resources.

Changes in bias between or among conditions of interest not only can obscure trends but also can lead to erroneous conclusions if the change in bias runs counter to the true trend being monitored. Suppose, for example, that a program for monitoring sea turtle abundances is concerned primarily with detecting population declines if they occur. Suppose further that the earliest efforts undercounted the turtles but that over time the turtle counters got progressively better (more experience, better techniques, less bias) or improved the methods originally used. Under such circumstances, a real decline in turtle populations might be masked by a progressive reduction in bias, causing population estimates to increase even though the turtle population was actually declining.

This dangerous failure of a monitoring protocol is difficult to avoid, because the biases of sampling techniques are generally not known and are essentially never quantified (see "Validation of Techniques," below). Furthermore, most conservation biologists are concerned primarily with population declines, and what little evidence exists suggests that present methods tend to undercount. Thus, future improvements in bias reduction are likely to shift population counts upward, although the true population trajectory will be unknown.

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Comparability

Comparability is the quality of two or more measurements that quantify exactly the same phenomenon. They may differ only in the conditions of interest, which in our case are attributes such as time, species, or habitat. Two measurements of identical or even zero bias may still be difficult to compare if conditions that are not of interest (i.e., nuisance variables) are allowed to vary. Suppose, for example, that one uses a relative-density estimate to determine the effect of clearcutting on population size of an endangered lizard. In this case, habitat (clearcut vs. old growth) is the only variable of interest. Abundances of the lizard are estimated first in the undisturbed forests and then in representative samples of clearcuts using exactly the same protocol. The measurements are similar, but not exactly *comparable*. Why? Because the two measurements were done at different times. Perhaps the lizards underwent a seasonal decline in activity during the sampling period (in which case the second estimate would be lower than the first, even if the average population densities were the same). Perhaps the weather was warmer (by chance) during the second set of measurements. Perhaps some eggs hatched during your sampling, causing young of the year to be present only in the second (clearcut) sample.

Now consider a long-term monitoring program. The variable of interest is long periods of time (short-term fluctuations are not of interest—a "nuisance"). In this case, the time of the surveys must be different, although dates can sometimes be matched by time of year. Is it sufficient to conduct the surveys on the same calendar dates? Probably not. In an El Niño year, for example, breeding might be advanced (or delayed) in comparison to other years, or July 10 to 25 may be hot one year and rainy the next, and so forth.

A vast array of considerations is required to ensure that various population-density estimates are comparable across years, sites, observers, species, and so forth. *Absolute population-density* estimates are, by definition, comparable across the various situations of interest, but *relative population-density* estimates will only be comparable if they are collected under conditions that are identical in all the nuisance factors that can bias an estimate (e.g., species, weather, time of year, habitat, animal size distribution, forest height, reproductive condition, body condition, satiety state, social status).

Another important factor is how the data that are collected will be used by the investigator. Chances are good that survey information provided to an environmental management agency will be used as a baseline for documenting future trends in abundance or biodiversity. That implies that different individuals will carry out future surveys. In addition, the habitat may have changed. Thus, population assessments must be independent of observer skill and habitat structure. Sequential species lists to be compared may bracket the arrival date of introduced or colonizing species. Thus, it is vital that the level of assurance that an undetected species was in truth absent be very high. Ideally, one would want to *quantify* the level of assurance. Quantifying the probability of failure to detect a species that is not yet known to have been introduced or to have colonized is not easy, so a more broadly applicable metric of survey intensity must be provided.

Suppose that compared to baseline data a subsequent survey documents a significant decline in a species of special concern. The environmental manager will no doubt want to know what caused the decline. Thus, the manager may also crave baseline data on causes of mortality, or quantification of natural mortality or growth rates, to which present values can be compared in order to assess the causes of the population decline. An investigator must ensure that the sampling protocol provides such data.

If the sampling goal is to answer a short-term scientific question, rather than to monitor a population in the long-term, then it is easier to ensure comparability of the samples. Short-term studies generally allow one to control closely the observers, habitats, protocols, and equipment used. By choosing the sampling periods artfully, a short-term study often allows the experimenter to control environmental factors such as weather, season, and time of day statistically. However, conservation action routinely relies on retrospective use of abundance measures from the scientific literature. For example, while a researcher may have only a short-term objective in mind for a particular box-turtle study, future box-turtle conservationists may have to rely on population-density estimates from the earlier study to assess subsequent population changes in the study area. The long-term utility of survey results will be extended and broadened if the monitoring method is not contingent upon the specific observers or the equipment used, nor on the habitats, weather, seasons, or times of day sampled.

Precision

Precision is the exactness with which a quantity is measured. The mathematical average of a large number of imprecise measurements provides a relatively precise estimator. Hence, large samples are routinely used to increase precision (although large samples do not reduce bias). Progressively larger samples are needed for incrementally smaller improvements in precision, so the cost of a monitoring program can be very sensitive to the degree of precision required. Sample sizes are often chosen to provide minimally acceptable confidence limits for current research needs. Unless one is clairvoyant, however, it will not be possible to know what confidence limits may be needed for future comparisons. Both long- and short-term informational needs should be considered when assessing precision needs.

Typically, precision requirements for management studies are more relaxed than those for scientific studies. Most environmental managers are eager to avoid catastrophic changes in reptile abundances but are unconcerned with annual fluctuations unrelated to long-term declines. If populations of a target species normally vary up to 30 percent in size from year to year, for example, a manager may prefer to ignore changes smaller than 50 percent. A short-term scientific study may require substantially greater precision. The required precision will depend on study objectives and will vary from species to species. Short-lived species will normally show greater variability in abundance than long-lived species such as turtles or crocodilians. Long-lived species typically exhibit high annual variation in fecundity and recruitment. Estimating reproductive parameters of long-lived species may require a large number of years or tolerance of low precision (wide confidence limits). That point highlights one difference between population density and other population values. Most of this section is phrased in terms of population density, but those pursuing other goals need to keep in mind the importance of making adjustments for sampling other quantities.

When assessing the relationship between precision and annual variability, it is helpful to discriminate between sampling variation and process variation. In essence, *sampling variation* is the "error" inherent in the method used to estimate any instantaneous quantity, usually abundance, whereas *process variation* is the change in true abundance over time or space. Both types of variation contribute to the variability recorded in density estimates collected over time. For example, the best estimates of the number of turtles in a pond over 3 years may be 105, 47, and 85 (variance = 868), whereas the true abundances are 91, 87, and 81 (variance = 25.3). Sampling error for the three years would be 14, −40, and 4 (variance = 825.3). In this example, sampling variation was responsible for most of the variation observed in the annual estimates; process variation was relatively slight. The sampling method chosen must have sufficient precision (low enough sampling variability) to detect the process variation of interest. A prospective power analysis (see "Precautions for Quantitative Reptile Field Studies," in Chapter 3) can be conducted if preliminary data upon which to base estimates of the sampling and process variation are available (for known measures of annual variation in estimates, see Gibbs et al. 1998, and related internet site, www.pwrc.usgs.gov/monmanual/samplesize.htm; a useful website for all population biology freeware is www.mbr-pwrc.usgs.gov/software.html.)

Efficiency

Efficiency is the benefit/cost ratio: (value of results obtained) / (cost of obtaining those results). Efficiency of reptile sampling is often (and improperly) measured by assessing just the denominator of the benefit/cost ratio: how much of "resource *x*" is required? The cost quantities most often measured are person-hours, elapsed time, money, specialized equipment, and environmental disruption (Gibbons and Semlitsch 1982; Bury and Raphael 1983; Sutton et al. 1999). Assuming that two competing methods provide the same degree of bias, precision, and comparability, aggregate cost is an appropriate metric of technique efficiency. Two methods are unlikely, however, to provide comparable benefits. Furthermore, benefits are rarely tabulated, as bias, precision, and comparability are difficult to quantify.

One indirect measure for the aggregate benefit of low bias, great precision, and high comparability is the number of individual reptiles detected or collected. Many investigators (e.g., Fitch 1992; Greenberg et al. 1994; Parmelee and Fitch 1995; Christiansen and Vandewalle 2000; Engelstoft and Ovaska 2000; Enge 2001) have compared the number of reptile individuals detected by competing methods. Pitfall-trapping/drift-fence arrays often yield high numbers of specimens. One pond with an encircling drift fence, for example, produced 52,287 emerging *Rana utricularia* [= *Lithobates sphenocephalus utricularius*] in a single season (Gibbons and Semlitsch 1982). Unfortunately, relative precision gains associated with such high sample numbers are of comparatively low value when estimating population sizes of species in which recruitment has extremely high process variation. Some species normally exhibit large swings in annual recruitment; consequently, the annual recruitment numbers are relatively poor long-term predictors of the fate of the population. Populations of species with more restricted recruitment tend to be more sensitive to the annual recruitment rate. Thus, a large number of individuals of one species is not equivalent to large numbers of other species. Yield is correlated with precision but is an incomplete proxy for the aggregate benefits of bias, precision, and comparability.

Validation of Techniques and Assumptions

A technique is valid for a specific application if it has the needed levels of bias (low enough), precision (high enough), and comparability (high enough). Scientists generally expect an assay to be validated. When a chemist analyzes the metal content of a sample of ore, he or she is expected to use a method that has been validated by calibration runs with ore of known concentration. Similarly, the test report from a medical laboratory that has diagnosed a blood sample should include a statement indicating the probabilities of obtaining false positives and false negatives. For some reason, however, we have no such expectations for validation of assays of animal populations. Even in the simplest case, a study to establish presence/absence, no established methodology for estimating the probabilities of obtaining false positives and false negatives exists.

A comparison among techniques, without quantifying bias, precision, and comparability, is called *soft validation*. A test that establishes the accuracy (precision + bias) of a technique is *hard validation*. A test to establish the validity of one aspect of a technique falls in between; perhaps we could describe it as a "moderate" validation.

Hard Validation

Population biology depends on accurate population estimation, but fewer than a dozen papers on hard validation of reptile inventory or monitoring techniques have been published. Let us consider seven of these.

Campbell (1996) reported that mark-recapture gave an unbiased and reasonably precise estimate of the number of Brown Treesnakes (*Boiga irregularis*) in two fenced 1-ha forested enclosures on Guam. Koper and Brooks (1998) tested a variety of methods for the analysis of mark-recapture data on a small, closed population (110 adults) of Painted Turtles (*Chrysemys picta*) in a small pond (1.7 ha). All methods significantly underestimated the true abundance of the turtles, often grossly so. Variation in detectability of individual turtles was apparently responsible for much of the bias. Koper and Brooks concluded that no known method was adequate for ecological research-related or management studies on this population.

Taylor and Winder (1997) placed five models of Sand Lizards (*Lacerta agilis*) along 100-m transects in suitable heath habitat in England. The authors did not indicate if that density is characteristic of natural Sand Lizard populations. All five lizard models were visible from the marked straight-line transect. Searchers were designated as "novice," "expert" (<2 y searching experience), or "professional" (>2 y experience). When searchers were confined to the transect line, no group spotted more than half of the models (average for professionals = <1.0 /transect; average for novice samplers about 0.7 / transect). When the professionals were free to deviate from the transect line, they found nearly all of them.

Experience was not statistically related to rates at which Desert Tortoise models and real scats were sighted in 1-ha natural habitats in California (Freilich and LaRue 1998). Sighting rates averaged about 60 percent for tortoises and 17 percent for scat. An artificially high density (10X natural density) of models was used in that study and in the study of Anderson et al. (2001), who used the same models. In both studies, the effects of observer experience were minimal. The sizes of the tortoise models (65-, 180-, and 290-mm carapace length, termed "juvenile," "subadult," and "adult" tortoises, respectively) strongly influenced the results of the Anderson study. Almost all (about 96%) of the adult tortoise models near the centerline were detected, but only about 80 percent of subadult models and less than half (44%) of the juveniles near the centerline were sighted.

Henke (1998) performed a hard validation test for searchers attempting to find some combination of Texas Horned Lizards (*Phrynosoma cornutum*), horned lizard scat, and *Pogonomyrmex* ant mounds in a Texas desert. The ant mounds in the area were devoid of vegetation, built of distinctive gravel particles, and averaged 0.87 m in diameter, making them relatively conspicuous. Thirteen ant mounds, 10 radio-collared lizards, and 5 to 50 placed scats were present in a 1.2-ha area, which was searched by groups of 10 individuals. Detection fraction was strongly dependent on the number of items being sought. If the searchers were asked to look for only one type of item, they found 73 to 82 percent of those items. If they were asked to search for two items, the yields ranged from 64 to 72 percent for each. An average of only 44 percent of the items were found when all three were sought simultaneously. In addition, Henke found an effect of target density: observers detected an average of about 78 percent of the scats when scat density was >30/ha; that figure fell to 48 percent at 20/ha, 37 percent at 10/ha, and 13 percent at 5/ha. Those observations indicate the potential for detection rates to be artificially elevated in studies using models or real animals at unnaturally high densities.

Brown Treesnakes and two species of geckos (*Hemidactylus frenatus* and *Lepidodactylus lugubris*) were grossly undersampled in forested 1-ha areas on Guam during hard validation of distance sampling (see "Transect Surveys, Including Line Distance," in Chapter 13; Rodda and Campbell 2002). The snake population estimate was low by a factor of about 7, and the gecko estimates were low by factors of about 18 (*H. frenatus*) and 148 (*L. lugubris*), respectively.

These few hard-validation studies could provide an adequate basis for inferring the validity of the tested techniques in the ecological systems where they were appraised, if they tested the full range of conditions under which the tested techniques would be used. They provide negligible insight into the validity of the techniques for other organisms and other systems, although they do suggest how a validation study can be carried out.

Designing a Hard Validation Study

A population of animals of known density is required for a validation study. The studies cited in the previous section used planted radio-telemetered animals, realistic animal models, or censused populations confined to a pond or fenced area (see Rodda, Campbell, and Fritts 2001). Models are much simpler to deploy than live animals, but they lack the realistic movements that attract observers and the evasive behavior that helps them to avoid detection. The use of models is, presumably, more of a concern for active, wary, or stealthy species (e.g., crocodilians) than for land tortoises. Detection of randomly placed models has been used to validate some studies, but the procedure may not be natural, because live animals may actively choose cryptic locations. Realistic density and placement of models is essential if the results are to be interpreted quantitatively. In my experience, sheen or gloss is a very important component of detection of cryptic species; models that are naturally placed and accurately colored but lack the appropriate surface finish could thus have an unnatural detection probability.

The known population of models or animals should be sampled with the test technique. The *test technique* is the technique to be validated; the *reference technique* is one that has been previously validated and whose precision is known. In the case of placed models or "planted" live animals (which are often radio-telemetered), the true number is known absolutely, so no reference sampling is needed. In the case of free-living animals, however, it may be necessary to use a high-validity reference technique to establish the "true" density. Often, scale is a problem when matching test and reference techniques. Line transects (a test technique), for example, require an extended sample area, whereas total removal plots (a reference technique) are necessarily very small, typically 10 × 10 m. To the extent of scale mismatch, the validation test depends on the representativeness of the reference plots. Care should be taken to distribute the samples in the most equivalent manner possible. The best population estimators from among the test techniques can then be compared to the reference technique with regard to bias, precision, and comparability.

Bias in this instance is the difference between the estimator obtained using the test technique and the estimator obtained using the reference technique, divided by the reference value; it is usually expressed as a percentage (two examples are given in the "Bias" section, above). Referencing the absolute bias within the confidence limits of an estimate is routine (e.g., a true value of 18/ha is within the confidence limits of 12–20/ha), but that practice overlooks bias for imprecise estimators and confounds bias and precision; if one's reference measure is imprecise, a test is uninformative. For absolute population-density estimates, the target is zero bias. For relative population measures, rigidly consistent proportional bias of any known amount may be acceptable. For example, suppose that observers always overlook 40 percent of a target species. As long as observers do not differ in this attribute, and the error rate is consistent across the conditions of interest (species, density, habitat, weather, etc.), the relative population measure is valid. Demonstrating that equivalence throughout the full range of conditions of interest can be a daunting task. Detection probability may be a nonlinear function of sampling conditions or population density. Determination of the full shape of the nonlinear function under all field conditions would require a very large number of validation tests. Furthermore, applying correction factors always widens the confidence limits that are associated with the definitive value. Consider, for example, a species with a detection probability of about 20 percent (SE = 8%) under rainy conditions and about 44 percent (SE = 12%) when sunny. Uncertainty associated with those correction factors (note standard errors) compounds with variation in the counts (confidence limits of estimator) multiplicatively to magnify the composite uncertainty about the population density. Removal of the sampling bias may be simpler and more informative than applying correction factors in some circumstances.

Precision is relatively easily measured by the width of the confidence intervals around the estimator. Repeated comparisons between test and reference estimators facilitate the dissection of the variance into sampling and process variation (see Thompson et al. 1998). Estimates of precision need to be regarded with some caution, as confidence limits are only as good as the assumptions built into the computational model. Koper and Brooks (1998) pointed out a variety of ways in which precision estimates can prove misleading.

Comparability depends on the uses to which the data will be put. For a long-term monitoring project, it would be necessary to show, for example, that different observers and habitats produce consistent, or at least quantifiable, bias. Published reports on comparability are limited, as comparability testing requires a vast array of comparisons for relative population measures (to my knowledge, none has been conducted for reptiles). Absolute population estimators, in contrast, are "absolute" because they are impervious to changing conditions, observers, etc. An absolute population estimator would be judged valid by showing acceptable levels of bias and precision.

Validation of Assumptions

Sometimes it is neither possible nor practical to validate a proposed technique. Likewise, some proposed techniques have been tested for validity and failed. In such cases, it is helpful to test the validity of the assumptions inherent in the technique in lieu of doing a full validation test. Such testing helps discern the cause of validation failures when they occur. The range of assumptions is so vast that it would be impossible to itemize all of the tests that one might propose. Some assumptions are limited to particular techniques (e.g., loss of marks is germane to mark-recapture but not to distance sampling). Nonetheless, at least five general classes of assumptions provide trouble for almost all population-sampling schemes.

Closure

Closure is a term used to describe a population when no individuals leave or enter the study area during the course of a sample. Animals may move around *within* a study area during a sampling period without violating closure, but animal movements that result in counting the same individual more than once will bias some population estimates, such as those based on distance sampling (see "Transect Surveys, Including Line Distance," in Chapter 13). Unless a sample is taken more or less instantaneously, however, some detectable animals may leave the detection area (emigrate from the sample) before being counted, or move and be double-counted. An unmarked lizard may flee from an approaching observer, for example, but eventually be spotted a second time in another location. Because the lizard is unmarked, the observer is likely to count the second sighting as a second lizard. With long-term studies, animals may enter and leave the study area during sampling, thereby inflating the pool of potentially detectable animals above the instantaneous population size. For example, we once removed 155 snakes during 15 days from a continuous forest area that averaged a population of only about 37 snakes at any one time (Rodda, Fritts, and Campbell 1999). Researchers may assume that few animals come and go if the habitat patch they are studying is isolated. Unfortunately, many reptiles slip across habitat discontinuities when researchers are not looking (see "Quadrat Sampling," in Chapter 13). Failure of assumed closure is rarely noticed unless the animals are marked. A typical mark-recapture manifestation is the failure, despite saturation marking, to obtain a population that is 100 percent marked. During an intensive mark-release program of garter snakes (*Thamnophis radix*) in an isolated swampy woodlot in the city of Chicago, investigators were unable to exceed a marked fraction of 14 percent over 2 years, despite marking hundreds of animals after the initial mark-out (Seibert and Hagen 1947; Seibert 1950). The assumption that the population was demographically closed does not seem plausible, despite the apparent isolation of the habitat. Although that is an extreme example, any asymptote short of 100 percent during continued marking indicates failure of closure. The program CAPTURE (http://www.mbr-pwrc.usgs.gov/software/capture.shtml) incorporates a mark-recapture closure test, but it is confounded by behavioral responses (e.g., trap shyness) and inconstancy of related phenomena (Otis et al. 1978), and like all statistical tests, it is weak when data are sparse (see also Stanley and Burnham 1999). Thus, failure to reject the null hypothesis in the CAPTURE closure test is only limited support for the closure assumption. The existence of a test for closure in mark-recapture studies should not mislead one into thinking that closure is a requirement only for mark-recapture studies; closure and response-to-observer violations can bias results of any technique.

Detections Proportional to Abundance

A vast number of reptile population studies assume trap or visual detections are proportional to the abundance of the species. Thus, twice as many detections of species A than of species B is assumed to indicate that A is twice as abundant as B. No evidence from the literature documents identical detection fractions for different species. Even closely related species differ in habitat use, behavior, and so forth. The validation data that have been collected suggest that the detection fraction depends not only on species but also on weather, habitat, age/size/developmental stage of the animal, stratum in the forest, reproductive condition, year, time of year, body condition, satiety, and social status (Table 14). Some techniques, especially open-model mark-recapture techniques, encompass the additional assumption that the detection probability of the animals that are detected is identical to that of individuals that *are not* detected. Although only some of the factors that influence detection will distort a given comparison, it would be a rare comparison that was not vulnerable to at least one of those sources of bias. Any valid monitoring program needs to demonstrate that these and other sources of bias are either negligible or controlled by the experimental design.

A special case of the proportionality assumption is the expectation that detections will be a linear function of abundance within a species. This assumption has rarely been tested, but evidence suggests that it fails in several respects. Henke (1998) suggested that search-image acquisition on the part of the searcher was facilitated at higher target densities, thereby explaining the proportionately greater detection rate at higher target densities (and a sigmoidal detection function in relation to density). The converse distortion (detection rates lower at higher target densities) can occur when there are

TABLE 14
Factors That Influence Reptile Detectability and Their Manifestations

Factor Type	*Manifestation*	*Source*
Species	Detection rates with species–barrier interaction	Brown (1997)
	Skink detection with species–weather interaction	Coddington and Cree (1997)
Weather	Sea turtle sightings disrupted by wind	Beavers and Ramsey (1998)
	Skink detection with species–weather interaction	Coddington and Cree (1997)
	Many species sensitive to weather conditions	Jones (1986)
Habitat	Crocodile sighting rates with body size–habitat interaction	Bayliss (1987)
	Lizards spp. with habitat–species interaction	Towns (1991)
	Snake detection rates habitat dependent	Rodda and Fritts (1992)
	Alligator detection dependent on water level	Wood et al. (1985)
Size of individual	Crocodile sighting rates with body size–habitat interaction	Bayliss (1987)
	Only large tortoises easily sighted	Anderson et al. (2001)
	Snake traps fail to capture small individuals	Rodda, Fritts, Clark, et al. (1999) Rodda et al. (2002)
Forest stratum	Day geckos with weather–perch height interaction	Gardner (1984)
	Snake sightings height dependent	Rodda (1992)
Reproductive condition	Pregnant female snakes oversampled	Bonnet and Naulleau (1996)
	Gravid female snakes undersampled	Rodda, Fritts, McCoid, and Campbell (1999)
	Detection of reptiles dependent on reproductive condition	Dunham et al. (1988)
Time-of-year	Detection of lizards dependent on time of year	Fitzgerald et al. (1999)
	Snake detectability dependent on time of year	May et al. (1996)
Body condition	Trap captures greater for underweight snakes	Gragg et al. (2007)
Satiety	Trap captures greater in low-prey environments	Gragg et al. (2007)
Social status	Subordinate male lizards undersampled	Andrews and Stamps (1994)

agonistic behavioral interactions among the targets, or the number of detections that can be processed simultaneously is limited. Andrews and Stamps (1994), for example, found that territory-holding *Anolis* lizards suppressed the locomotor activity of subordinate individuals. Thus, with increasing population density, trap-capture yields (or sightings) would be expected to reach an asymptote when most potential territories were occupied. Single-capture traps or visual sightings that need to be recorded while nearby animals are slinking away can produce the same nonlinearity. Multiple-capture traps may produce the opposite effect if animals are drawn to the trap by the presence of captive conspecifics.

All Individuals in a Specified Area Are Detected

Distance sampling, line transects, and many presence/absence studies make the assumption that every individual in an intensively searched area has been detected. Validation studies such as those reported above (see "Hard Validation") cast considerable doubt on this assumption. Detection probabilities can be quantified by mark-recapture in some cases, but the approach is impractical for assessing the detection probability of rare or potentially absent species. Managers often solicit complete faunal inventories on the assumption that presence/absence information is easy and therefore less expensive to obtain than are monitoring data (which include abundance assessments). This assumption needs to be challenged by highlighting the scientific difficulties of inferring absence from nondetection (see "Occupancy," under "Point Estimates," in Chapter 15).

Interobserver Consistency

All techniques rely on the skill of the person doing the searches, setting the traps, and so forth. Even purely mechanical traps may produce much greater numbers of captures when managed with an artful touch (Enge 2001). Visual searches may be extremely sensitive to observer skill (Rodda and Fritts 1992; Rodda 1993; May et al. 1996). In many cases, investigators do not even balance the representation of different searchers in different treatments, because such a balance may be logistically or financially expensive. Studies that demonstrate an absence of effect of searcher skill (e.g., Freilich and LaRue 1998; Anderson et al. 2001) are helpful in such situations, although failure to find a statistical effect can sometimes indicate only that the sample size was inadequate. A prospective power analysis can be used to indicate the magnitude of the process variation in comparison to the sampling variation induced by interobserver differences.

Area Sampled Is Representative of the Population of Interest

It is generally impractical to study an entire area of interest. More often, one samples. The external validity of a study is then dependent on the degree to which the sample reflects the larger population of interest. In some cases (for example, mark-recapture), one may be uncertain as to the area occupied by the population sampled. Animals travel beyond the confines of a trap area or sampling plot. For small plots, the area of uncertainty may be large in relation to the area directly sampled; in one well-studied reptile case, the sampled area was larger than the trap grid area by a factor of more than three (Mitchell 1999). For population density estimation, accurate estimation of the area sampled may be as important as documentation of the representativeness of the sample area.

The credibility of a population-sampling technique depends on the extent to which the technique's assumptions are valid. Testing of assumptions is an essential part of science, yet to date few such tests have been performed for reptile sampling. If an investigator can validate a technique with a relevant study, he or she can be confident that the work not only produced a valid monitoring result for the species but also has advanced the field of herpetological population biology.

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CHAPTER THIRTEEN

Standard Techniques for Inventory and Monitoring

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Selecting a Technique

Gordon H. Rodda and Craig Guyer

Eleven standard methods for use in reptile inventory and monitoring projects are presented in this chapter. They were selected because they are broadly applicable to the diverse array of lineages comprising the group Reptilia as well as to the extensive array of habitat types that reptiles occupy. These methods will be useful for various types of inventory and monitoring projects. However, we remind investigators initiating studies that they must invest sufficient time and effort in the design phase of the study to ensure that the procedures they select are appropriate for their particular projects.

The authors begin their presentations of each standard method with a summary of the background literature that has led to the development of the method in its present form. This is followed by a description of the target organisms and habitats for which the method is most appropriate. Finally, the research design, field methods, personnel, and materials required to implement the method in a variety of settings are given. Each technique is narrowly defined in order to avoid problems that arise when characterizations of a particular technique are so broad that they conflate key attributes. For example, to some people a "visual encounter" survey means general herpetological collecting (i.e., wandering through a forest and disrupting reptile refugia), whereas to others it means a carefully bounded nighttime transect. Some investigators equate a quadrat survey with raking litter, whereas others equate the term with any survey of a bounded area. Variation in the definitions of these terms is important because changes in meaning are accompanied by changes in the assumptions that constrain our ability to interpret data from inventory and monitoring projects. This illustrates our need for clear, standard definitions of techniques to ensure accurate communication about our work. Knowledge of the assumptions inherent in any technique (and/or study) is vital for selecting study methods and interpreting data gathered with those methods. In this section we suggest a system for determining the key assumptions associated with the design of an inventory or monitoring project and highlight cases in which the assumptions may or may not match the project goals of enumerating the reptile species and the number of individuals of each that are present.

Selection from among the proposed standard techniques requires careful evaluation of the objectives of the study, timeframe of the work, and resources available to ensure that the assumptions associated with a particular method are appropriate for reaching the overall goal(s) of the study. It is particularly important that the design of an inventory or monitoring project accommodate the assumptions of the enumeration method to be used. Assumptions associated with an inventory or monitoring project are related to eight key features of the study: (1) taxonomic scope, (2) geographic scope, (3) temporal scope, (4) desired resolution, (5) detection technique, (6) concentration technique, (7) sample closure, and (8) population closure (Table 15). Choices associated with each of these features carry explicit assumptions. Investigators should strive to

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TABLE 15

Key Features Associated with Projects for Inventorying and Monitoring Reptiles[a]

TAXONOMIC SCOPE	GEOGRAPHIC SCOPE	TEMPORAL SCOPE	DESIRED RESOLUTION	DETECTION TECHNIQUE	CONCENTRATION TECHNIQUE	SAMPLE CLOSURE	POPULATION CLOSURE
Subset(s) of one species (e.g., males)	*One site within a habitat*	*Single time*	*Presence/Absence*	*Visual and/or auditory sample; no habitat disruption*	*None*	*None*	*None*
Detection probability of each individual in each subset is equivalent	Site located at random within habitat = representative of the habitat, or maximizes detection probability	Time selected to maximize detection probability. Seasonal trends not interpreted	Detection probability of each species or individual is equivalent	Detection probability of each species or individual is equivalent			
One species	*Multiple sites within a habitat*	*Across time*	*Relative abundance*	*Visual and/or auditory sample habitat disrupted*	*Physical, chemical, or auditory attractant*	*Transect*	*Extrapolation from depletion rate*
Detection probability of each individual of the species is equivalent	Sites located at random within habitat = representative of the habitat, or maximizes detection probability	Sampled at fixed or random intervals	Detection probability constant over time interval of interest and equivalent for all species or individuals	Detection probability of each species or individual is equivalent; refugia known and sampled at random	Enhancement rearranges distributions of individuals but alters abundance; all individuals attracted equally; all species equally trappable	Transects sample species and habitats at random	Detection probability constant over time interval of interest and equivalent for all species or individuals

Multiple species	*One entire habitat*		*Absolute abundance*	*Trap- or net-assisted sample*		*Trapping webs*	*Closed-model mark-recapture*
Detection probability of each individual within each species is equivalent	Individual organisms or species sampled at random or censused		Animals censused, or detection probability of all individuals equivalent	Detection probability of each species or individual equivalent		Web covers habitats in proportion to their abundance; trappability constant for each individual	No migration; no reproduction; no marks lost; detection probability constant over time interval of interest
	Multiple habitats					*Quadrats*	*Open-model mark-recapture*
	Samples at random within habitats; habitats sampled in proportion to their abundance; relative representation of habitats does not change over time					Animals censused	No marks lost; Detection probability constant over time interval of interest. Detection probability constant for each individual
	Rangewide						
	Species sampled at random across range						

a. Below each feature is a set of alternative states (in italics) among which an investigator chooses before starting a project. The major assumptions of each alternative state (no italics) follow.

design studies that meet these assumptions. However, researchers studying reptiles rarely test these assumptions; if data from a future study demonstrate that an explicit assumption(s) was violated, they may be used to falsify the results of that particular earlier study.

Each of the features listed above encompasses a continuum of possibilities; we have grouped them into a series of discrete categories that cover the situations typical of reptile studies:

> *Taxonomic scope* generally conforms to one of three categories: intraspecific (e.g., focus on a particular sex or age group), target specific (on a single focal species), and faunal (on all members of a supraspecific taxonomic group or the entire assemblage of reptiles at a site).
>
> *Geographic scope* has two components. One is the planned landscape level, which ranges from a single habitat at a particular site to all habitats across the entire geographic range of a species or herpetofaunal assemblage. The other is the planned sample intensity; it ranges from a single representative sample to replicate samples.
>
> *Temporal scope* refers to the number of samples and their timing. It can range from a single sampling event to repeated sampling events designed to characterize changes across time.
>
> *Resolution* refers to the level of detail in the data required to meet the goal(s) of the study; it ranges from a simple list of the presence or absence of species to estimates of the numbers of individuals of each species and the variances of those estimates.
>
> *Detection technique* refers to the way that individual organisms will be discovered and, therefore, the level of disturbance to the habitat that it will incur. Techniques range from simple visual encounters of exposed individuals, which cause negligible disturbance, through various kinds of trapping, to active alteration of the environment to discover or trap hidden individuals.
>
> *Concentration techniques* are those that direct or attract individuals to a study area and the degree to which they do so. Methods range from those in which no direction or attraction occurs to those in which physical items (e.g., drift fences or cover boards), chemicals (e.g., prey odors), or auditory signals (e.g., mating vocalizations) cause individuals to aggregate.
>
> *Sample closure* refers to the degree to which the sample area is known. Methods range from those in which the sample area is unknown (e.g., haphazard searches), through those in which the boundaries of the sample area are fuzzy (e.g., trapping webs, pitfall arrays), to methods in which the sample boundary is fixed (e.g., quadrats).
>
> *Population closure* refers to the degree to which the area from which individuals are sampled is controlled. Immigration, emigration, mortality, and reproduction can all occur in an open population in the study area, and the number of individuals in the population is dynamic. In contrast, migration, mortality, and reproduction cannot occur in a closed population in the study area, so the number of individuals in the population is static.

Two examples illustrate how selection of categories within these features can clarify the assumptions that must be made and identify problems that may arise during a particular study. Suppose, for example, that investigators decide to use the road-cruising technique (see "Road Riding," below) to quantify the abundance of a single snake species relative to other snake species, in a particular area, over several years. Such a study might be classified as follows:

> *Taxonomic scope*: single species
>
> *Geographic scope*: multiple habitats
>
> *Temporal scope*: across time
>
> *Desired resolution*: relative abundance
>
> *Detection technique*: visual
>
> *Concentration technique*: attractant (i.e., the road)
>
> *Sample closure*: none
>
> *Population closure*: none

No special assumptions are involved in the taxonomic scope. The presence of multiple habitats along the road (and the roadway itself), in conjunction with the desire to describe abundance for the entire sample area, requires an assumption that the detection probability is constant among any different habitats through which the road passes. Otherwise, the sample will be biased by overweighting counts from the habitats for which detection is easiest. The temporal scope of the study, in combination with the multiple habitat feature, forces the assumption that the relative representation of the habitats does not change over sampling time (e.g., if a farmer cuts down a stand of trees after the first year of study, the relative proportion of each habitat has changed). The objective of obtaining only relative abundance eliminates some restrictive assumptions about detection probabilities (the probability of detecting an individual does not have to be any particular value, as it would if absolute abundance were the objective); however, the requirement to estimate abundances over time forces the assumption that the detection probability (whatever it may be) is constant over the time interval of interest (i.e., the probability of detecting individual organisms is the same in each year and in each season sampled). The latter assumption is highly unlikely given that the occurrence of snakes on a road is highly seasonal and dependent on weather conditions. The absence of sample closure is problematic because the area represented by the observations cannot be determined. Therefore, any differences observed among years may be due either to changes in species richness, changes in numbers of individuals observed among years, or changes in the activity patterns that alter the distances over which individuals travel and, therefore, their probability of encountering the road. The absence of population closure means that there will be no way to verify that detection probability is constant over any time interval or geographic space of interest. In particular, since the road does not provide a random sample of the region, the data cannot be extrapolated over a broader landscape.

Contrast the list of assumptions above with the smaller set indicated for a mark-recapture study of the adult sex ratio of an aquatic turtle in a pond. Such a study might be classified as follows:

> *Taxonomic scope*: multiple strata (in this case adult males and females) within a species
>
> *Geographic scope*: one habitat
>
> *Temporal scope*: single time

TABLE 16

Key Characteristics of Standard Techniques for Inventorying and Monitoring of Reptiles[a]

Standard Technique	*Taxonomic Scope*	*Sample/ Census*	*Data Resolution*[b]	*Effort to Establish*	*Equipment Required*
Complete inventory	All species	Sample	P/A; RA	Large	Extensive
Road riding	Nocturnal snakes	Sample	P/A; RA	Small	Moderate
Visual encounter	All species	Sample	P/A; RA	Small	None
Quadrat	Small, leaf-litter squamates	Census	AA	Large	None
Mark-recapture	Single species	Census	AA	Small	None
Transect	All species	Sample	AA	Large	Moderate
Pitfall traps	Mobile terrestrial/aquatic	Sample	P/A; RA	Large	Extensive
Artificial cover	Secretive squamates	Sample	P/A; RA	Large	Moderate
Reptile sign[c]	Mobile terrestrial[d]	Sample	P/A; RA	Large	Moderate
Nest and track	Large turtles/crocodilians	Sample	P/A; RA	Small	None
Aerial survey	Large turtles/crocodilians	Census	AA	Large	Extensive

a. Selection of techniques can be guided by study features selected from Table 15.

b. Desired resolution of population data: P/A = presence/absence; RA = relative abundance; AA = absolute abundance.

c. Photographs from camera traps, track stations, burrows, etc.

d. Especially species using loose substrates, creating structures (e.g., burrows), etc.

Desired resolution: absolute abundance

Detection technique: net-assisted sample

Concentration technique: none

Sample closure: area fixed (census)

Population closure: closed

In this case the assumptions are less troubling. The interest in multiple strata within the population requires the assumption that all strata of interest (males and females in this case) are equally detectable or that the detectability within each stratum can be estimated separately (e.g., with mark-recapture). Limiting the project scope to a single habitat at a single time eliminates the need for assumptions about detectability in different habitats and at different times. Although an investigator may be satisfied with data describing relative abundance, it is impossible to obtain a valid estimate of sex ratio without knowing the relative detectabilities of the two sexes; consequently, estimates of absolute abundance must be obtained because only methods associated with this level of resolution will provide these measures of detectability. It may appear that using a net to collect the animals requires an assumption that the net collects each sex equally, but because detectability is estimated separately for each sex, no such assumption need be made. The pond habitat has a discrete boundary, so the assumption of sample closure is not troubling. However, the basic assumptions of mark-recapture (population closure, no marks lost, all marks read without error) are critical.

To assist the researcher with the selection of techniques from among the eleven standard methods, we summarize five key features of each (Table 16). Three of the methods are broad enough to be of use for a wide variety of taxa (complete inventory, visual encounter, transects). The other methods are generally restricted to specific subsets of reptiles. The costs associated with each method also differ, which is always an important issue in field studies. One set of costs is associated with the degree of data resolution desired. In general, the time required to determine absolute abundance is much greater than that required to determine presence/absence or relative abundance, which inflates costs. A second set of costs involves the time and effort required to establish a particular sampling method in the field. Typically, if set-up efforts are substantial, field crews with several personnel are required. They, in turn, will need considerable time for building or assembling devices for concentrating animals (e.g., cover boards, drift fences, aquatic traps), as well as maps on which to base sampling regimes. In addition, extensive base-camp infrastructure (e.g., established field stations or semipermanent field camps) will be needed for the crew, for processing specimens, and so forth. Single investigators generally can establish sampling methods requiring little effort, with limited preparation time. A third set of costs involves specialized equipment required to implement a particular method. Costs associated with techniques that require special modes of transport (e.g., aerial surveys), established trapping stations (e.g., drift fences or cover boards), or precise placement and measurement of sample locations (e.g., transects) can be moderate or extensive. By careful evaluation of the information in tables 15 and 16, investigators can select methods that achieve their study goals in a cost-effective manner.

Complete Species Inventories

Christopher J. Raxworthy, Natalia Ananjeva, and Nikolai L. Orlov

One of the desired objectives of many reptile surveys is to record all species that occur in the survey area. This goal leads to the obvious question, however, of how one will know when the inventory is complete. In reality, although most reptile inventories strive to be complete, few researchers are able to support this claim convincingly. Nevertheless, simple techniques and approaches can be utilized during the inventory work that will greatly enhance the completeness of the survey. Many of these approaches may seem obvious, yet it is surprising how often species inventories are seriously compromised by a few poor decisions that were made in the field. Common mistakes include surveying at the wrong time of the year, insufficient night work, and not recognizing important local habitat variation, such as on elevation gradients.

Complete or nearly complete site inventories are needed in order to describe community structure accurately, which may be crucial to ecological studies, biogeographic analyses, or habitat or species management. Species inventories are also being used increasingly as a basis for setting conservation priorities, so the completeness of a survey could even have political and economic ramifications. Because time and financial support are always limited, however, most surveys must strive for completeness under highly constrained conditions (e.g., see Boxes 6–8 for examples). Thus, complete inventories of all but the simplest of reptile communities are usually unobtainable. However, all surveys yield species locality data, and modern techniques utilizing ecological niche modeling approaches are able to generate predictive maps of species distributions. This approach can be used to help set conservation priorities (e.g. Kremen et al. 2008), guide inventory work to regions likely to yield new species (Raxworthy et al. 2003), and in the future may be able to help supplement incomplete inventories for sites.

Target Groups

The techniques discussed in this section target all reptile species in a study area, although there is a greater emphasis given here to terrestrial species. Aquatic species (especially sea turtles, sea snakes, and crocodilians) have their own suite of specialized survey techniques that are discussed in detail elsewhere in this volume.

Background

It is important to distinguish species inventories, which generate lists of species for sites or regions, from others types of surveys that record either relative species richness or the abundance of individuals. For example, the Systematic Sampling Survey (SSS) approach described by Scott (1994) for amphibians can be equally well applied to reptiles, if the major goal is to compare species richness between sites or to monitor for changes in species richness over time. However, if the goal is to detect the largest number of species in the least amount of time, the SSS approach will not be as efficient as would be general collecting by experienced collectors (Scott 1994). For this reason, because this chapter focuses on complete species inventories, general collecting techniques for reptiles are emphasized here.

Complete species inventories of reptile communities are costly in terms of time and other resources. Consequently, for species-rich reptile communities, few such inventories have been completed to date, with the major exceptions being long-term surveys that are centered at research stations such as La Selva, Costa Rica (Guyer 1990), and on Barro Colorado Island, Panama (Rand and Myers 1990). By contrast, many sites and regions have been intensely surveyed for shorter durations, and these studies have yielded species lists that report a substantial component of the reptile fauna. Examples of such surveys include Bas López (1982), Brown et al. (1996, 2000), Ferner et al. (2001), Ineich, (2003), Nussbaum et al. (1999), Raselimanana et al. (2000), Raxworthy and Nussbaum (1994, 1996); Vega-López and Alvarez (1992).

Research Design

The geographic or spatial scope of an all-species inventory must be determined before any claim to completeness can be made. The scope may be defined by the boundaries of a protected area or other political unit, or by a natural landscape feature such as a habitat patch. Spatial scope must also be carefully considered from the perspective of the resources available for the proposed survey. Species inventories are normally conducted at the alpha diversity level, that is, the species richness of ecosystems. These studies may be based on single sites or on transects that span an environmental gradient such as elevation. However, larger-scale, beta-diversity inventories consider regional species richness and compile results from multiple sites and sources. These beta studies will, of course, include greater species richness, which can give the impression of greater completeness of species sampling. Yet, if there is substantial turnover of species within the region being considered, the opposite case may be true. This situation is especially common in the tropics, where reptiles may show considerable local endemism.

Temporal scale is also important. Over both ecological and evolutionary time, species distributions change; thus, inventory lists for a site can also be expected to change with time. Investigators involved in long-term inventories may assume that a reptile community remains unchanged during the survey period, but in fact, local populations may have gone extinct, or new species may have dispersed into the study area. The assumption of a temporally static reptile community, therefore, also needs to be carefully considered during the interpretation of inventory results.

Finally, complete inventories need to be designed from two perspectives: (1) the general survey techniques needed to sample an entire community, and (2) the quantitative approaches required to assess the completeness of the survey.

Field Methods

Many methodological factors will influence the completeness of an inventory (Table 17). The most important requirement for a complete inventory is that the entire range of habitats represented within the inventory area be sampled. Reptiles are impressive for the diversity of habitats that they occupy and the habitat specializations that many species exhibit. Important features to consider when identifying habitats are vegetation type, substrate type (especially rock, soil, sand), elevation, slope aspect, humidity, and water availability. Among the factors most commonly neglected are elevation in the tropics and substrate type in arid regions. An investigator moving a few hundred meters up or down an elevation transect or onto a new substrate may encounter previously unrecorded species. For tropical regions we recommend having survey sites separated by no more than 300 m elevation wherever possible.

BOX 6 SPECIES INVENTORIES IN CENTRAL ASIAN DESERTS

Natalia Ananjeva and Nicolai L. Orlov

Field Sites Desert regions with varied types of vegetation, habitats, and topographic features that have vehicle access. Prior to fieldwork, regional and taxonomic experts are consulted about logistics and the natural history of the area.

Field Methods Radial routes 3- to 5-km long are used to survey areas surrounding camp. Searches are conducted in all available habitats, including canyons, sand dunes, and oases, and at all elevations. At the beginning of the survey, the extent of each microhabitat type is estimated and then each microhabitat is surveyed. Searches are carried out at 100-m intervals along roads during the morning, and at the beginning, middle, and end of the night. Drift fences (see "Pitfall-Trap Surveys," below) are erected in habitats considered typical for potential species. Fences are linear or circular around a specific habitat. Fences (30-cm high by 50-m long) typically consist of plastic sheeting attached to pegs at 1- to 1.5-m intervals, with the lower edge buried in the ground. Pitfall traps (cylinders or plastic buckets buried flush into the ground) are placed at intervals along the drift fences. Channels are sometimes dug to guide animals into the pitfall traps. Plots (25 × 25 m) fenced as described above are used to sample select communities (especially of diurnal lizards) in selected microhabitats. Camera and video traps are sometimes set (depending on vulnerability to theft) to record lizards in places of expected basking or foraging. Each site is sampled for 2 weeks.

Sampling is conducted in different seasons, from early spring (the onset of reptile activity) until late autumn (when activity wanes). Variation in seasonal activity of reptiles among habitats and landscapes is taken into consideration during survey planning. Nearly complete inventories require repeated sampling at different times of the day (and night), repeated sampling in different seasons, and repeated sampling over 3 to 5 years; even then, some rare fossorial species of reptiles may not be recorded. Local people are regularly asked about the presence of particular reptiles and shown their photographs; specimens of particular species may be purchased subsequently.

Field assistants include experienced technicians and biology students from local universities. A team of five is used to search plots (25 × 25 m), while canyon surveys require two to three researchers. Local guides and hunters, with knowledge of local species, join the field excursions to search for rarer species.

Logistic constraints are usually the main reason why some habitat types are poorly surveyed by inventory work, but this can be offset by careful planning and preparation.

Microhabitats and refugia are also important to reptiles. Typically, knowledge of species' microhabitat specializations can only be gained from local experience, and without that experience, it is frustratingly easy to miss species. Collaborating with local reptile specialists is key in this regard. Examples of potentially important microhabitats include vegetation strata, root mat, leaf litter, tunnels and burrows, ant and termite nests, cavities in dead wood, leaf axils, caves, rock crevices, and areas under bark, rocks, debris, and other materials.

Seasonality represents another critical aspect of complete surveys. Almost all reptile species are seasonally active, becoming torpid during either cold (hibernation) or dry (estivation) periods. Inactive reptiles are almost impossible to survey because they typically occupy inaccessible or highly cryptic refuges; thus, surveys must be timed to coincide with periods of activity. Unfortunately, the period of peak activity can differ among reptile species within the same site or region, making no single time ideal for sampling all species. Consequently, the complete survey of a site may require that it be visited during multiple seasons. As a general rule, the wettest months are the best for arid-climate species and the warmest months the best for humid-area species. In addition to activity, other seasonal factors must be considered carefully. For example, vegetation cover can be important (e.g., savanna species can be more difficult to find during periods when the grass is tall), as can access to remote sites, which may be impossible to reach during peak rainy periods. Local field experience is almost always needed to determine optimum survey periods, although regional climate data will also provide valuable information for scheduling surveys. Unfortunately, many reptile inventories are not conducted during optimal periods, due to conflicts including school semesters, project deadlines, and the availability of logistical support.

Survey effort has a major impact on the completeness of a survey, particularly with regard to species that occur at low densities or are distributed in areas that are difficult to sample. Snakes and fossorial species are typically the least abundant or most cryptic component of a reptile fauna and, thus, the most vulnerable to undersampling. The number of singleton

TABLE 17

Survey Features That Influence Inventory Completeness

Survey Feature	*Actions That Improve Inventory Completeness*
Vegetation types	All types considered; vegetation maps and remotely sensed images consulted
Substrate types	All types considered, including rock formations, sand dunes, river sediments; geological maps and remotely sensed images consulted
Landscape features	All types considered, including caves, water bodies, river drainages, ridges, cliffs, canyons
Elevation	Entire elevation range considered; sample bands span <300 m elevation
Slope aspects	All aspects included, and basking opportunities considered
Microhabitats and refugia	All types considered, including root mats, leaf litter, burrows, deadwood, leaf axils, and rock crevices, as well as under bark, rocks, debris
Habitat degradation	Both pristine and degraded habitats surveyed; local knowledge of habitat history considered
Seasonality	Surveys conducted in all seasons, or otherwise in the wettest and warmest periods of the year
Daily survey schedule	All time periods surveyed, including at night
Survey duration	Duration of time surveys based on species accumulation curves
Survey team size	Team size maximized to fit logistic constraints
Survey team field experience	Team experience maximized, while allowing for training opportunities
Survey team motivation	Survey designed to maintain team motivation (e.g., by providing adequate resources, field breaks, etc.)
Trapping methods	Diversity of trapping methods maximized, and new methods included where possible
Local knowledge	Local specialists and local communities consulted
Museum collections	Museum catalogues searched for records of specimens collected from the survey region; information used to guide and/or supplement fieldwork
Published literature and online resources	Literature searched for reports of specimens or habitats; information used to guide and/or supplement fieldwork

species (species represented by just one specimen or observation) is a good index of the magnitude of sampling problems in the field. Singleton species normally belong to taxa that would benefit from increased survey effort. Inventory effort can be readily increased either by including more people or traps in the survey or by extending the period of sampling. It is important to stress, however, that reptile surveying is very much influenced by the experience (and motivation) of the team members; thus, the total number of participants in a survey is not, in itself, an adequate measure of survey effort.

The choice of trapping methods depends heavily on conditions at the sites to be surveyed and the reptiles expected to be found. Experienced field herpetologists have their own preferred techniques, depending on local collecting conditions. As a general rule, using a high diversity of trapping techniques allows one to evaluate trapping efficiency and to sample a broad range of taxa. Field techniques for the capture of live animals include seizing with the hand (or snake tongs), rubber-banding, noosing, digging, and trapping (e.g. sticky traps, terrestrial and aquatic funnel traps, basking traps for aquatic turtles, pitfall traps with or without a drift fence, artificial refuge traps such as tin sheeting, mats, and nets; see Chapter 5, “Finding and Capturing Reptiles”). Specimens intended for preservation can be collected with an air-pellet gun or a handgun with dust (#12 pellets) shot. A vehicle is also a valuable collecting tool. Vehicle cruising (see “Road Riding,” below) is an efficient way to collect certain cryptic or rare species, because huge transect distances can be covered over short periods. Road kills (especially snakes) and active nocturnal and diurnal species (especially turtles and larger lizards and snakes) can usually be spotted easily on roads or tracks. The best time to survey for road kills is often at dawn, before scavenging birds find them. Specific trapping techniques are explained in further detail elsewhere in this volume (see Chapters 5 and 11, and the rest of this chapter).

Efficient use of many collecting methods (e.g., rubber-banding, noosing, trapping) requires practice. Other techniques are still being discovered or refined. For example, laser pointers have recently been used to lure geckos down from walls! Other researchers use blowguns with darts or balls of clay, or sticks wrapped at the end with duct tape. Nooses can be used to capture animals in crevices if the noose line (just adjacent to the noose) is threaded through a narrow, flexible copper tube.

Almost all trapping methods work best when animals are most active. This means that capturing nocturnal reptiles (a component of almost all reptile communities) requires significant sampling efforts at night. Ideally, survey work should be conducted at all times of the day and night, to ensure that all periods of potential activity are represented. Under hot conditions, diurnal species may show a bimodal peak activity, with

BOX 7 SPECIES INVENTORIES IN SOUTHEAST ASIA

Natalia Ananjeva and Nicolai L. Orlov

Field Sites A vehicle is used to locate primary and secondary forests at different elevations, and sites are selected for sampling based on the availability of local habitats. Each elevation zone is sampled for from 15 to 60 days.

Field Methods Sampling routes extend radially from the camp. Different habitats and microhabitats are selected for diurnal and nocturnal investigation. Streams, canyon slopes, paths, groups of giant trees (for access into the canopy with climbing equipment), and relatively flat sites covered with deciduous leaf litter are typically included. Woven mats of fine bamboo or thick plastic are placed on the forest floor in the flat areas; later, they are quickly lifted for sampling of the leaf litter and fossorial species hiding underneath. These mats can also be used as clean surfaces for hand searching leaf litter from the forest floor for small reptiles. Pitfall traps (buckets or cones) are placed every 5 to 6 m along trenches dug into compact ground to a depth of from 50 to 60 cm. Trenches are checked at night, and any potential communication tunnels that may indicate the presence of fossorial reptiles are investigated. Fine-mesh nets are also used for catching agamid lizards (*Bronchocela, Calotes,* and *Acanthosaura*). Nets are stretched between trees or bushes, wound around the trunks of the trees, or attached to poles for sampling arboreal species.

Diurnal surveys (as well as habitat photography and reconnaissance for night excursions) are typically made between 14:00 and 17:00 hours. Typical night excursions last from 18:00 to 24:00 hours or from 03:00 to 05:00 hours and cover a distance of 500 to 2,000 m, depending on the topography. Most reptiles are active during the first hours after dusk, but some are more active just prior to dawn (e.g., the rare snake *Azemiops feae*). Night sampling usually ends after a route has been searched from 15 to 60 times.

After arriving at a new site, we meet with local people, including hunters. Using photographs of reptiles, we obtain information on the potential presence of different species, with special focus on rare species and their habitats. During the subsequent reconnaissance, local hunters make survey routes that include identified habitats of interest. The most intense survey efforts are usually needed to record fossorial forms (Uropeltidae, Typhlopidae, Cylindrophiidae), secretive species (*Opisthotropis* spp., *Tropidophorus* spp., and some *Sphenomorphus* spp.), and canopy-inhabiting forms (arboreal agamids, skinks, small geckos, and arboreal snakes of the genera *Boiga* and *Pareas*). Local guides, students, and zoologists from local universities help with all aspects of fieldwork. When a site is to be visited repeatedly, local people are asked to continue searching even when the main scientific team is absent; this increases the likelihood of finding secretive species, and species with short activity periods.

animals inactive during the hottest period of the day. Otherwise, activity may peak at midday, and there may be no activity if overcast or raining. Few methods are designed for capturing inactive or torpid animals, because discovering (e.g., underground) or accessing (e.g., deep rock crevices) refuges is difficult (but see Chapter 11). In tropical forests, “smoking” will drive specimens from hollow trees, and the cruel practice (which we strongly oppose) of pouring gasoline into hibernation dens has been used to drive out rattlesnakes.

Tapping into local knowledge is also valuable for surveying certain species. For example, local individuals may be aware of the locations of certain habitats that are otherwise not obvious (e.g., caves, ponds, relict forests), or they may have had direct experience with local reptiles and be able to recognize their tracks and burrows or to point out areas where they occur. This is particularly the case for nesting sea turtles and crocodilians. Local fishermen often are familiar with the habits of aquatic turtles, and fish markets can yield important discoveries. It is also important for investigators to introduce themselves to and consult with local communities if they plan to carry out any surveys (especially at night) in close proximity to houses or areas of special sensitivity or significance (e.g., protected areas or sacred sites).

Finally, a review of the published literature and a search of relevant museum collections (see Appendix I) will enhance the completeness of a survey. Some museums are likely to have specimen material of interest, and the institutional locations of collections are also typically reported in the literature. In all instances, however, the identifications of museum voucher specimens and the associated locality data must be carefully verified. Museum specimens have the exciting potential to reveal interesting temporal changes in reptile distributions

BOX 8 SPECIES INVENTORIES IN MADAGASCAR

Christopher J. Raxworthy

Field Sites Areas with natural vegetation or unusual geology or topology, protected areas, areas that have not been surveyed previously or have only been partially surveyed, areas with exceptional elevational range, areas with rare or transitional vegetation types, and areas where species of interest have been collected previously are usually selected as field sites. Access is by vehicle or walking (up to 4 days). Survey camps are established as close as possible to the area to be surveyed and occupied for from 5 to 30 days (typically 5–7). Usually two to five survey camps are established during the visit to a region or protected area.

Field Methods Searches are confined to areas within half a day's travel from the camp. Diurnal surveys are usually carried out between 08:00 and 17:00 hours and nocturnal surveys between 19:00 and 24:00 hours. All habitats and microhabitats are surveyed, with care taken to include the complete range of elevations and vegetation types available. Local landscape features such as rivers, streams, waterfalls, caves, canyons, rock outcrops, rock fields, and sand dunes are explored. Primary and secondary vegetation and human-modified areas (including villages) are surveyed, although effort is concentrated in primary vegetation. Most survey work is confined to the rainy season (November–April). Data from previously collected museum specimens and the published literature are used to supplement field data. Completeness of the survey is assessed using species accumulation curves and proportion of singleton species.

Trapping methods include hand capture, rubber banding, digging, and pitfall traps with drift fences. Pitfall trapping follows a standard design (Raxworthy and Nussbaum 1994): the pitfall traps are buckets (275 mm deep, 290 mm top internal diameter, 220 mm bottom internal diameter) with the handles removed and small holes (2-mm diameter) punched in the bottom to allow water drainage. Buckets are sunk into the ground along a drift fence made from plastic sheeting (0.5 m high) stapled in a vertical position to thin wooden stakes, with the fence bottom sealed 50 mm deep into the ground using soil and leaf litter. Trap lines are checked each morning and late afternoon. After rain, the buckets are sponge-dried. The drift fence (100 m long) is positioned to run across the middle of each pitfall trap. Pitfall traps are positioned at both ends of the drift fence, with the other nine traps at 10-m intervals. Three lines are typically used in the following forest types: ridge (along the crest of a ridge), slope (half way between the ridge top and valley bottom), and valley (within 20 m of a stream in a valley bottom).

The survey team includes Malagasy graduate students, research assistants, and local guides. Typically there are four to six experienced workers. Visits are made to local communities and to the appropriate authorities to obtain/present authorizations, explain the purpose of the survey, and describe techniques to be used. At the same time, we request location information for sacred or sensitive sites to be avoided, local *fadys* (taboos) to be respected, sites of potential research interest, the presence of certain well-known species (e.g., crocodiles, tortoises, aquatic skinks), and any prior history of research or exploitation.

and can also help to direct fieldwork to either poorly surveyed areas or areas with interesting species requiring modern verification.

Personnel and Materials Required

The experience of the survey team and the duration of the survey are critical factors to the completeness of the inventory. Reptile inventory work usually involves teams of at least two experienced herpetologists, ideally with other team members contributing to the field effort, who also receive training and field experience. The duration of time spent sampling a site is discussed in more detail below. The diversity of sampling methods available for reptiles means that each survey will have its own unique requirements for materials. If the survey is being conducted in a remote site where resupply is not an option, a degree of redundancy in the materials is important. This will allow for contingencies of equipment failure or loss.

Data Treatment and Interpretation

The decision to terminate survey work at a site and move on to a subsequent site is crucial because field time is highly constrained by the availability of resources and logistic sup-

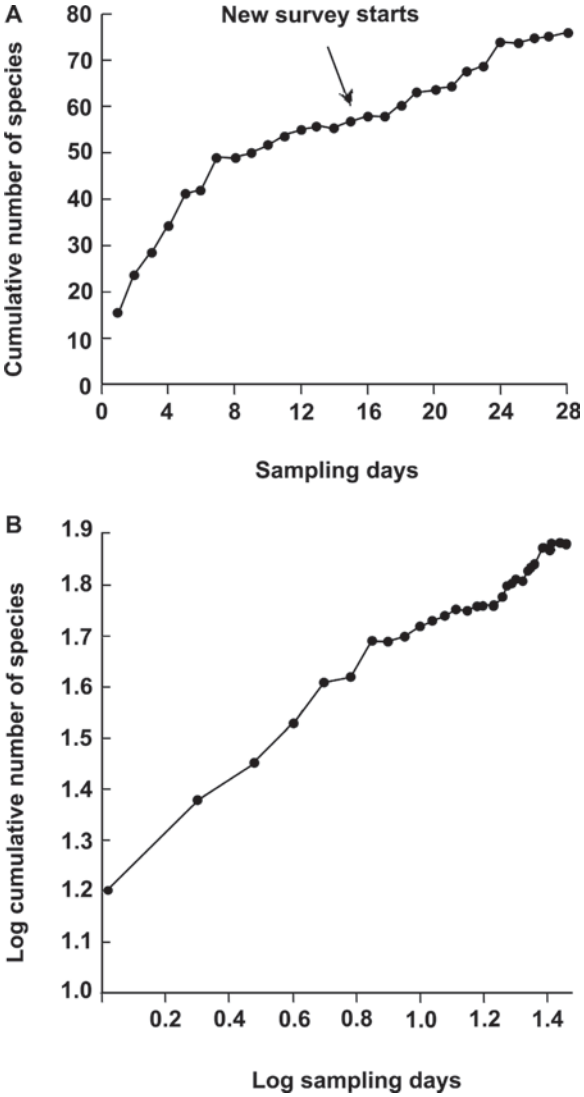


FIGURE 70 Species accumulations for amphibians and reptiles at a single survey site at Marojejy, Madagascar (based on Raselimanana et al. 2000). (A) Normal accumulation curve plot. An asymptote appears close to being reached during the final four survey days. Note also an indication of an earlier asymptote, prior to the start of the second phase of the survey. (B) Log-log plot of the same accumulation data. Note how the accumulation curve has now been transformed into a straight line.

port. In many cases, completeness of a survey at a single site must be weighed against the need to maximize efficiency of surveying species over a larger region, which requires collecting data from additional sites.

Inventory completeness at a site is commonly determined using species accumulation curves. These graphs plot total species accumulated against survey time (days, months, or years). During the early phase of a survey, the curve climbs steeply, but then it gradually flattens out to reach an asymptote. The asymptote is usually interpreted to mean that all (or almost all) of the species present have been "accumulated" (i.e., recorded; Fig. 70A). The length of the period required to reach an asymptote depends on how rapidly species are accumulated during the earlier phases of the survey. For intense survey efforts of very small areas, an asymptote can be reached after only a few days. More typically, however, considerably more time (weeks to years) will be required. Plotting species accumulation curves is an attractive way to document progress during the course of an inventory. In practice, however, most researchers intuitively terminate surveys once they recognize that few or no additional species are being added to the inventory, despite ongoing intensive survey effort.

Unfortunately, accumulation curves can also be misleading by underestimating actual species diversity. This occurs when areas are surveyed during suboptimal periods or with suboptimal methods. For example, dry-season surveys of reptiles produce asymptotic accumulation curves because many inactive species remain undetected. In such cases, asymptotes obviously would not be good measures of total species diversity. Another problem is that long term, large-scale inventories of diverse communities may produce species accumulation curves that never reach an asymptote (Rosenzweig 1995, pp. 63–67). This is unlikely to be a problem for reptile communities, however, except at massive spatial scales. Either semilogarithmic or log-log plots of species-time curves produce straight-line relationships (Fig 70B).

Another practical way to assess the completeness of a survey is with measures of *species equitability,* or variation in species' abundances. Species represented by just a single capture (or observation) provide a warning that other species with similar habits may not yet have been detected. These "singleton" species are unlikely to be rare; rather, the low capture rate usually reflects problems of sampling. Arboreal and fossorial species (especially snakes) are among those most often represented by single specimens.

Equitability and accumulation curve criteria can be combined to develop *stop rules,* which determine when to terminate sampling. For example, sampling could terminate when no additional species have been found in 10 days (*asymptote duration*) and all species are represented by at least two specimens. Appropriate stop rules can be developed by changing either the asymptote duration and/or the minimum number of captures of the rarest species.

Special Considerations

In some cases it may be obvious that an inventory is far from complete. That is typically the case when a survey is short and sample sizes are small. Nevertheless, it may still be possible to compare diversity measures with those of other better-studied sites, provided that the sampling methods are comparable (i.e., the same trapping methods and protocols have been used). Although species richness at one site might be clearly incomplete, other indexes of diversity, such as those that combine both species diversity and measures of equitability, can be used. Two commonly used indices are Fisher's α and Simpson's Index of Concentration. The applications of both are reviewed by Rosenzweig (1995, pp. 192–204). Standardized short-term sampling techniques can also be used to rank species richness between sites, using methods such as the Systematic Sampling Survey (SSS) approach described by Scott (1994).

Road Riding

Brian K. Sullivan

The thrill of this collecting lies in the white crinkly appearance of a real snake suddenly coming within the range of the

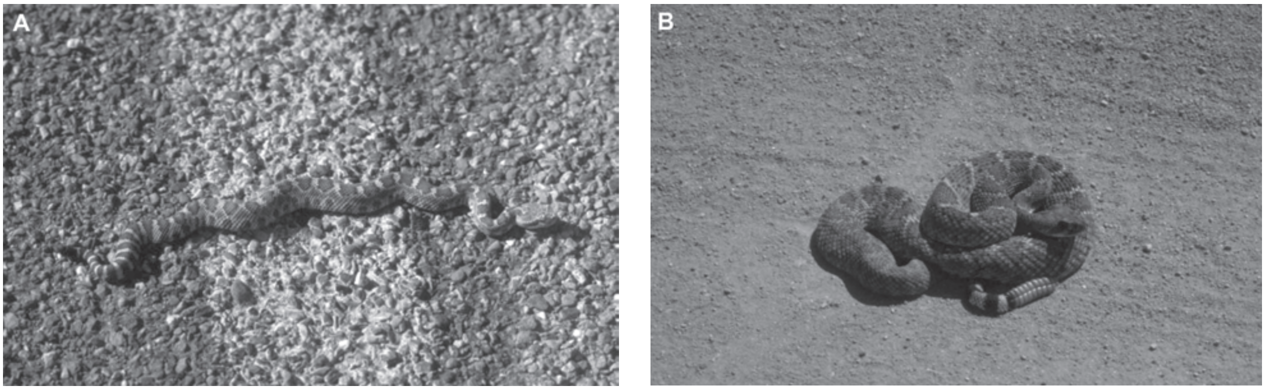


FIGURE 71 Snakes often bask on roads. A. *Crotalus oreganus* on a paved road. B. *Crotalus atrox* on an unsurfaced road. (Photos by B. K. Sullivan.)

headlights . . . it brings you out again after many a fruitless night. (L. M. KLAUBER, 1939)

Since the 1920s, it has been recognized that many reptiles, especially snakes, can be encountered by driving a motor vehicle slowly on a paved road during the early evening in spring and summer (e.g., Klauber 1931, 1939; Bugbee 1945; Fitch 1949; Hensley 1950; Campbell 1953, 1956). When a reptile is spotted, the driver stops the car, and a colleague jumps from the vehicle to grab or pin it for examination or collection. In a number of studies of snake communities, road riding has been the only sampling technique used, with paved roads functioning as "transects" through the habitat (Klauber 1939; Pough 1966; Sullivan 1981a, 2000; Mendelson and Jennings 1992). In addition, many collectors of reptiles and amphibians use road riding to obtain specimens for the pet trade and other purposes (Price 1985; Sullivan 2000).

Target Organisms and Habitats

Most road-riding studies (e.g., Klauber 1931; Pough 1966; Sullivan 1981a; Price and LaPointe 1990; Rosen and Lowe 1994) have focused on snakes of arid and semi-arid regions. Snakes in such regions appear to use paved road surfaces, which retain heat, for thermoregulation in the early evening during spring and summer; they also bask on both surfaced and unsurfaced roadways during daylight hours (Fig. 71). Investigators have also used the technique with snakes of more mesic temperate environments (Dodd et al. 1989; Bernardino and Dalrymple 1992). Bernardino and Dalrymple (1992) suggested that snake mortality on roadways in the Everglades, Florida, peaked during the dry season as snakes moved in response to fluctuations in water levels, which influence the distribution of prey and availability of refuge sites. Snakes are also encountered in large numbers on roadways in such environments during heavy rains.

Road-riders have also regularly noted lizards, especially geckos, in road-riding surveys. Klauber (1939) observed that many typically diurnal, desert lizards (e.g., *Callisaurus, Dipsosaurus, Sceloporus*) can occasionally be found on roadways at night, but not regularly enough to make this a profitable sampling methodology. Geckos (*Coleonyx, Nephrurus*), and even Flap-footed Lizards (Pygopodidae), are commonly collected using road riding in arid regions of North America and Australia (R. Shine, pers. comm.; BKS, pers. observ.). On a little-used road in the Sonoran Desert of central Arizona, I observed close to 50 Western Banded Geckos (*Coleonyx variegatus*) in less than 1 hour of road riding just after sunset after a brief summer rainstorm (BKS, pers. observ.). Road riding can also be used to survey diurnal lizards basking on the pavement or roadside berm in the early morning in open, arid environments (A. Muth, pers. comm.; BKS, pers. observ.). In a situation similar to that of the banded geckos noted above, I observed more than 40 adult Desert Spiny Lizards (*Sceloporus magister*) immediately prior to dusk along the pavement edge of a road in the Sonoran Desert west of Phoenix, Arizona (BKS, pers. observ.). It appears that the occurrence of turtles on roadways is too unpredictable for this technique to be an efficient method for surveying them (but see Ashley and Robinson 1996).

Background

Klauber (1931) was the first to publish a detailed analysis of road riding as a sampling method for reptiles, specifically snakes; his work remains the definitive treatment of the technique (see also Klauber 1939). In pioneering reports, he summarized results from 7 years (1924–1930) of road driving in coastal scrub and desert scrub habitats throughout San Diego and Imperial counties in southern California. In 402 trips, he traveled 28,813 miles and observed 755 snakes dead on the road (DOR) and 97 alive on the road (AOR).

Klauber (1939) considered a variety of "factors involved in night collecting." He suggested that spring is the best time for collecting snakes in both coastal and desert habitats, noting a drop in numbers in summer. He encountered snakes at the highest rate in May (0.045/mi of road), followed by June (0.042/mi) and April (0.037/mi). There was a minor peak in the encounter rate in the fall. Klauber also found that collecting was better on nights with little wind or moonlight, but the negative correlation between these variables and snake numbers was weak. He suggested that snake mortality is greater in areas where the density of vegetation bordering roads is low. Finally, Klauber (1931) suggested that nocturnal snakes are active throughout the night, although in some temperate environments observations of most snake species decline 2 to 3 hours after sunset.

Klauber compared road riding with hunting snakes on foot (at night) and concluded that road riding was more productive. R. A. Seigel (pers. comm.), who surveyed the herpetological communities of the relatively mesic, temperate environments of central Florida, found that road riding was an efficient and effective means of inventorying the diversity of reptiles and amphibians when compared with limited-area searching, pitfall-trap arrays, cover boards, and other techniques.

Mendelson and Jennings (1992) used road riding to survey two roads in southeastern Arizona and southwestern New Mexico that had been sampled by road riding roughly 30 years previously (Pough 1966). They documented significant shifts in relative abundance of some taxa, and interestingly, found many small, secretive forms that Pough did not. Mendelson and Jennings suggested that they may have detected small snakes more effectively than did Pough but did not specify differences in sampling methodologies that might explain this result. Langen et al. (2007) documented that small snakes were missed during vehicular surveys but detected during pedestrian surveys along the same route in New York, lending support to the notion that detection of small individuals can vary considerably depending on the survey methods and personnel. Sullivan (1981a, 2000) used road riding to survey the distribution and abundance of snakes across an ecotone in central California and, like Mendelson and Jennings (1992), noted a shift in community structure over 20 years.

Research Design and Field Methods

An ideal sampling method for assessing diversity and abundance would detect all target organisms of interest (e.g., an entire snake community) in direct proportion to their representation in the associated habitat. Clearly, road riding will not do this, because it does not sample reptiles randomly within a given habitat (e.g., see Klauber 1939; Sullivan 1981a; Price and LaPointe1990; Mendelson and Jennings 1992). Specific biases associated with road riding undoubtedly are directly tied to the taxa surveyed. First, it is widely assumed that large snakes (i.e., individuals >250 mm total length) are more likely than small snakes to be detected on road surfaces; this seems reasonable, although the lighting, vehicle speed, and visual acuity of the surveyor undoubtedly are also important, especially in the detection of small individuals. Those with extensive experience in road riding can attest to their ability to detect snakes on roadways missed by other passengers in the same car and other surveyors in separate vehicles (BKS, pers. observ.). Second, species undoubtedly vary in their propensity to venture onto roads. Price and LaPointe (1990) and Sullivan (2000) all noted that small secretive snakes (e.g., *Diadophis, Sonora*) known to be abundant in the habitat immediately adjacent to the roadway were rarely detected in road-riding surveys. Andrews and Gibbons (2005) demonstrated that snake species differ in their reaction to the exposed nature of a roadway surface: smaller species were less inclined to cross. They also found that snakes vary in their response to approaching vehicles; those that "freeze" have a higher likelihood of being struck by a vehicle while crossing a roadway. Third, the lengths of time that snakes remain on a road surface may vary. It has been commonly noted that certain snakes appear to use the road surface actively to thermoregulate (e.g., Sullivan 1981b). Although the extent to which this behavior may increase the likelihood of their detection is unknown, it clearly increases their detection probability relative to taxa that simply cross the roadway. Certain taxa (*Crotalus, Lampropeltis, Pituophis*) are numerically dominant in roadway surveys in the United States (Klauber 1931; Sullivan 1981a, 2000; Price and LaPointe 1990; Mendelson and Jennings 1992); although these species are no doubt abundant in many habitats, they are also the snakes commonly observed actively thermoregulating on the road (Sullivan 1981b). Finally, snakes may learn to avoid roads, an especially troubling consideration for long-term monitoring using road riding and for studies in which sites are revisited (e.g., Mendelson and Jennings 1992; Sullivan 2000).

Dodd et al. (1989) discussed factors that might influence the numbers of reptiles seen on roads and found that distance driven and rainfall were positively correlated with numbers of snakes observed. They also questioned the utility of using road running for diversity estimates after encountering only 11 of 22 species of small, terrestrial snakes known from their survey area. They argued that comparisons among road-running studies are not valid because techniques used differ, as do the snake species being sampled. However, Jones et al. (2011) obtained nearly identical results at two sites in the Sonoran Desert of Arizona, with road riding, suggesting that the method does provide consistent results for at least some snake communities.

Although Hensley (1950) noted an extreme male bias in the sex ratio of DOR snakes that he collected in southwestern Arizona, it is not clear that this bias was a result of using road riding as a sampling technique. Because male snakes move long distances when searching for mates during the breeding season, it is reasonable to assume that they might be encountered more readily with this method than with another technique with which active snakes were encountered on a natural surface. Coleman et al. (2008) documented seasonal shifts in numbers of DOR reptiles, with higher numbers during the spring and fall activity periods (see Reynolds 1982). They also documented higher numbers of DOR specimens as vehicular use of the roadway increased.

These observations suggest that investigators will be most successful road riding on dark, windless nights during the peak activity period of the target organisms. Road riding is easily employed and may be relatively efficient given the labor-intensive efforts associated with field studies of many squamates. Nonetheless, like all techniques, it has limitations, and the notion that over time snakes may alter their behavior and be less inclined to venture onto a paved road, especially if such behavioral shifts vary across taxa, may compromise investigations using road riding exclusively.

Personnel and Materials

Minimally, a single individual and a motor vehicle are required for this technique. The approach varies depending on preferences of the individual, but most workers drive along a paved roadway between 10 and 40 mph (16–64 kph) during the early evening in the activity season of the target organisms. Klauber's (1939) suggestions for employing this technique are widely regarded as appropriate today. He suggested that if safe, one should drive in the middle of the road so as to illuminate as much of the pavement as possible, using "low beams" ("city lights"); running lights or other means of enhancing the view of the road are no doubt useful, but local driving statutes may restrict the use of lights over and above headlamps. A vehicle that provides the driver with a clear view of the roadway is efficacious, as is ease of vehicle access.

Klauber (1939) also evaluated the method with regard to the physical characteristics of the road. He suggested that heavily traveled roads be avoided because of the increased mortality of the snakes and safety concerns. He also noted that it may be difficult to observe animals among the debris on such roads and that heavily traveled roads often possess "improved" shoulders or berms that may discourage reptiles from entering the roadway. Finally, he suggested that paved roads are better than unpaved for surveying, perhaps because blacktop paving holds heat better and longer than dirt. Although I am unaware of any controlled studies to test the effects of these variables, most surveyors who regularly use road riding as a sampling technique would likely agree with Klauber's recommendations.

Road riding can take two forms: *dedicated road riding,* as described above, in which one drives along a stretch of roadway exclusively in the hope of observing AOR snakes, or *incidental road riding,* in which one uses the method passively by simply noting snakes observed while traveling between two points (e.g., Klauber 1932). In the latter instance, primarily road-killed (DOR) organisms, which document species presence and mortality, are recorded.

The habits and habitats of the target organisms will largely determine the effectiveness of the road-riding technique. As described above, most studies have focused on arid- and semi-arid-region snakes that are nocturnally active during warm weather; under these circumstances, road riding would be most profitable during the late afternoon and early evening. However, if diurnally active forms are of interest, then early-morning surveys may also be profitable. Some vertebrates use culverts to avoid crossing a roadway directly (Woltz et al. 2008), so investigators should avoid roadways with ample opportunity for such "undetected" crossings. The immediate microhabitat associated with the roadway edge will also influence the number of snakes observed. In the southwestern United States, rock-dwelling forms (e.g., *Trimorphodon, Hypsiglena*) are often found on roadways through rocky canyons with cliff faces immediately adjacent to the road. Langen et al. (2007) documented "hotspots" for various species along roadways they surveyed in New York.

Special Considerations

One critical concern is the extent to which roadways impact the ecology not only of reptiles to be surveyed but also the entire biotic community adjacent to the roadway. Roadways alter the hydrology, chemistry, physical structure, and other properties of habitats in many ways (Garland and Bradley 1984; Artz 1989; Spellerberg 1998; Terranella et al. 1999; de Maynadier and Hunter 2000; Trombulak and Frissell 2000). Delgado Garcia et al. (2007) showed that roadways facilitate connectedness of lizard populations in forested habitats of the Canary Islands. One lacertid was more common in the altered habitat along roadway edges than in undisturbed areas, which allowed for gene flow among populations previously isolated by unfavorable forest communities. If local amphibian and reptile communities change significantly in response to road-generated habitat alterations, then a road-riding survey may not accurately reflect the true community associated with the overall habitat through which the road passes. The deaths on roads of snakes and other organisms, including their prey, greatly affect the population biology of many taxa within a local biotic community (Fahrig et al. 1995; Seigel et al. 1998; Spellerberg 1998; Trombulak and Frissell 2000). Radio-tracking studies of Gray Ratsnakes (*Pantherophis spiloides*; Row et al. 2007) and Eastern Indigo Snakes (*Drymarchon couperi*; Hyslop et al. 2009) suggested that roadway mortality can significantly affect viability of small populations of threatened or endangered snake species. Overall, it is reasonable to infer that reptile communities in the immediate vicinity of roadways will be altered over time depending in part on traffic volumes and other local variables (Klauber 1939; Bernardino and Dalrymple 1992; Rosen and Lowe 1994; Spellerberg 1998; Jones et al., 2011).

Observations indicated that Common Gartersnakes (*Thamnophis sirtalis*) avoid roads and cross in the most direct path if crossing is necessary (Shine et al. 2004). If snakes do learn to avoid roads, then those behavioral shifts will compromise long-term surveys using road riding. In addition, if researchers use roadway surveys to estimate mortality effects at the population level, they must recognize that scavengers (e.g., ravens, vultures, coyotes) systematically patrol paved roads in the southwestern United States searching for carrion. Antworth et al. (2005), for example, found that from 60 to 97 percent of dead snakes placed on a roadway were removed within 36 hours by scavengers. While conducting road-riding surveys in central California, I often observed vultures and ravens feeding on snakes, and on one occasion I witnessed a raven removing a still writhing Common Kingsnake (*Lampropeltis getula*) within seconds after the snake was hit by a vehicle (Sullivan 2000). Thus, the number of DOR specimens recorded in a road-riding survey may dramatically underestimate mortality for local populations.

In summary, biases associated with road riding include the following: (1) species are not sampled equally (common to many techniques); (2) roadways alter the habitat by modifying the local hydrology and increasing the mortality of reptiles and their prey (the magnitude and nature of habitat changes may vary with the particular settings, i.e., road type and use, habitat, and reptile community); (3) reptiles may alter their behavior in response to the presence of roadways such that surveys on roads of different "ages" yield different results.

Visual Encounter Surveys

Craig Guyer and Maureen A. Donnelly

Visual encounter surveys (VESs) are used principally to inventory taxa present at a particular site. When broadly defined, a VES may employ any method, because humans tend to be visually oriented creatures. Here, however, we define VES narrowly, as employing only those methods most efficient for creating a list of species present at a specified site. We exclude closely related methods, such as quadrat sampling and transect surveys (covered in other sections of this chapter, below), that are more focused on assessing abundances of each species. In this section we describe the principal types of VES, discuss the situations for which each method is best suited, and identify the statistical treatments appropriate for analyzing data obtained with each method.

A VES is carried out by an investigator(s) who traverses a study area and records the identities of taxa as they (or their signs) are encountered. The investigator records the time and a description of the location of each observation and estimates the numbers of individuals of each species observed. Such samples are essentially dimensionless in that the specific sample localities and the time spent sampling the area are not specified with great precision. Rather, the investigator selects a general area for an inventory, which takes place as time permits. Sampling localities within the study area are selected because they are logistically feasible (e.g., accessible via an existing trail) and conform to the investigator's intuition regarding where reptiles are likely to be found. Typically, time spent searching is recorded roughly, for example, as the number of person-days over which a VES is conducted. Consequently, the only data sets that are produced by a VES in its simplest form are lists of species known to be present in the study area and a collection of field notes describing

ing the conditions under which each individual reptile was discovered.

An investigator can greatly enhance the utility of his or her VES data by recording the time spent searching (start and stop time for each sampling period), the distance surveyed (tracing the route traveled), or both, more precisely. In addition, if multiple investigators travel the same route, variation in the detectabilities of the species encountered can be evaluated. Information on these variables allows an investigator to go beyond a simple species list and use the data from the VES to provide measures of relative abundances and habitat selection. Armed with such information, the investigator can generate models that estimate the probability that a particular species occupies a particular site and then estimate the number of species present in the study site that were not detected (Tyre et al. 2003).

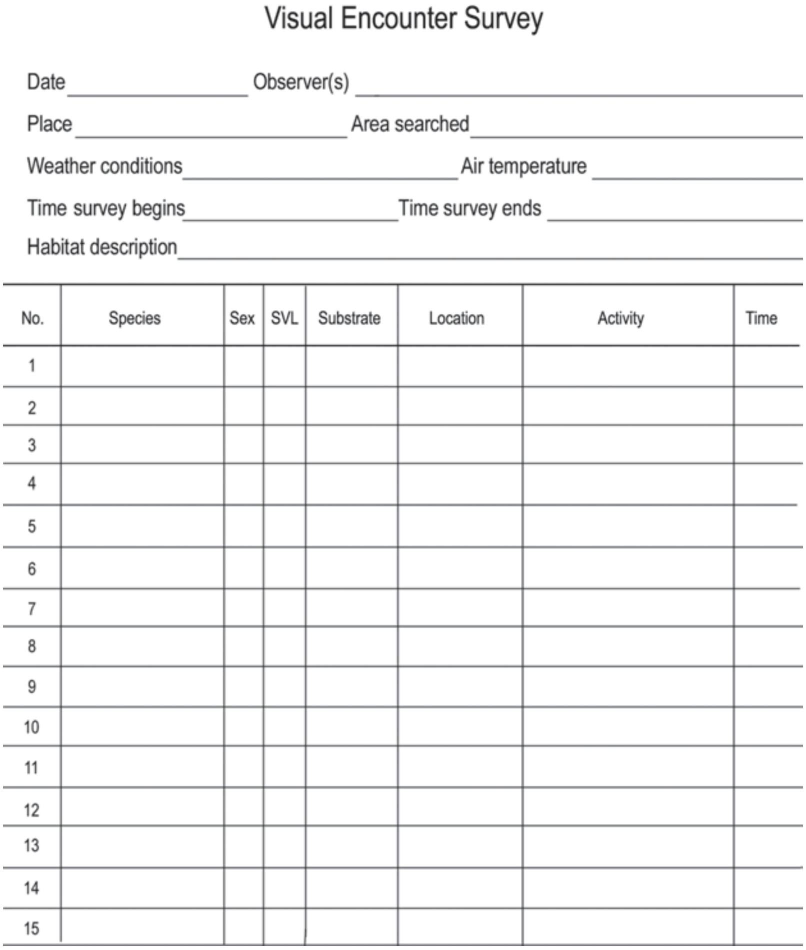
VES is best used for preliminary exploration of an unstudied area or an area for which the time available for study is restricted. If time is severely constrained, VES will be the only sampling tool available. This is particularly true of sites that are about to be altered by human activities (e.g., clear cutting a forest). The method has been used extensively in *Rapid Assessment Programs* (RAPs), which consist of surveys conducted over a very short period within a particular area; comparisons of the biodiversity within such areas (along with other factors) are then used to set priorities among the areas for protection through conservation efforts (Abate 1992). Researchers often use data gathered with VESs to inform the selection of more analytically rigorous methods for additional sampling. To maximize the utility of VES data, investigators should follow procedures that will increase the probability that all species present will be discovered. Although the term VES implies that vision is the principal mechanism by which a species is discovered, it does not mean that only animals active on the surface and, therefore, exposed to a visually-oriented investigator are recorded. Rather, investigators are expected to search all areas where potential members of a community are likely to be found. They must turn over surface debris, rake around the bases of buttressed trees, dissect epiphytic plants, examine recently fallen trees, follow tracks in loose soil, float or snorkel in lakes and rivers, and employ other, more specialized methods (see Chapter 5, "Finding and Capturing Reptiles," and Chapter 11, "Techniques for Reptiles in Difficult-to-Sample Habitats"). VES sampling is an active rather than a passive process. Investigators choose to search particular areas based on intuition and field experience. Frequently, local naturalists (i.e., individuals who have grown up exploring local habitats and encountering local fauna) have the best field intuition and, therefore, are among the best candidates for field crews.

Typically, investigators use existing trails to access study sites and sample day and night at temperatures at which reptiles are likely to be active (e.g., Lillywhite 1982). They should also create trails where none exists to allow access to all habitat types. Topographic maps of the site and other sources of geographical data (e.g., GIS plots and aerial imagery) can be used to determine where habitat types are likely to be located and to ensure adequate coverage of the study area. Such measures will increase the likelihood that all the reptile species in the area will be encountered. However, researchers interested in determining relative abundances of species should avoid sampling from the trails themselves because individuals may be more abundant or more detectable along trails than off of trails and the strength of the trail effect may differ among habitat types (von May and Donnelly 2009). Because reptiles often have distinctive patterns of seasonal activity, VES should be performed at least once in all seasons likely to be of importance to the local reptile fauna (e.g., spring and fall for temperate areas; wet and dry seasons for many tropical sites). Where possible, replicate samples should be taken so that variation within and among years can be documented.

Two modifications of the basic VES design are used. In *time-constrained searches* (Campbell and Christman 1982; Corn and Bury 1990), the time spent searching is regulated so that each habitat is searched for a period of time that is proportional to the availability of that habitat in the study area. In *distance-constrained searches,* the distance explored within each habitat is proportional to the available area of that habitat (transect design of Crump and Scott 1994; strip transects of Thompson et al. 1998). In both instances, efforts are made to avoid resampling the same areas within a habitat during a given sample so that individual organisms are not recorded more than once. Permanent transect lines can be established in each habitat and sampled repeatedly, or new transect lines can be established in each habitat at each sample period.

The primary assumption underlying VES is that individual reptiles are identified without error. Obviously, those carrying out the survey at a particular site must know the local fauna. Training investigators to recognize the species that they are likely to encounter prior to initiating a VES is helpful. The alternative is to capture a representative(s) of each species so that its identity can be determined (or confirmed). Field guides may be available for the local fauna so that personnel can identify unknown organisms by reference to pictures or by locating key identifying features that are described. In areas for which there are no guides, collectors can identify voucher specimens using scientific keys. If identification is not immediately possible, field personnel can sort the taxa into recognizable categories and refer to each with its own descriptive name (e.g., red-spotted purple lizard or round-headed, blue snake) until the species can be identified (e.g., Oliver and Beattie 1993). A field crew should, however, include someone competent to recognize taxa that may be new to science. In any case, vouchers (either scientific research specimens or high-quality photographic images) of all species discovered in VES studies should be prepared (Vitt and de la Torre 1996). Identifications can then be provided or confirmed by trained authorities or by comparisons with specimens in museum collections. Vouchering ensures that the data will meet the highest possible scientific standards (see Chapter 6, "Voucher Specimens").

VESs can provide essential qualitative data for the reptile fauna at a particular site. In order to maximize the utility of such searches, consistent information should be collected for each observation (Fig. 72). Time, date, and a description of the habitat should be recorded each time an individual is observed. Additional information on the size, sex, and activity of the individual should also be noted. Through accumulation of such field data, preliminary descriptions of the life history of each species can be generated (e.g., Vitt et al. 2002). Such data frequently are the principal source of information summarized in field guides (e.g., Guyer and Donnelly 2005), and publication of such guides should be an ultimate goal of projects in areas that are being surveyed for the first time. In order to maximize the utility of VES surveys, raw data (field notes) should be deposited in a form and at a place that makes them available to the scientific community for further study. It is often helpful to archive them at the institution in which



Visual Encounter Survey

Date ________________ Observer(s) ____

Place ________________________ Area searched ____________________________

Weather conditions ________________________ Air temperature __________________

Time survey begins __________________ Time survey ends ____________________

Habitat description ____

No.	Species	Sex	SVL	Substrate	Location	Activity	Time
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							

FIGURE 72 Sample data sheet for a Visual Encounter Survey. Use of data sheets helps to ensure that the information collected for each individual encountered is consistent and complete. (From Crump and Scott 1994; redrawn with permission.)

the voucher material documenting species' presences has been deposited.

Statistical analyses of data from VESs typically are limited because the methods are designed to determine presence or absence of species rather than their abundances. However, if certain key assumptions are met, then species accumulation curves (to decide when a fauna has been sampled adequately), rarefaction or its alternatives (to compare species richness among habitats or areas), or relative abundance can be analyzed (see "Species Accumulation Curves" and "Rarefaction," in Chapter 14). The key assumptions are that (1) all individual reptiles of all species are equally likely to be encountered in all habitats, (2) each individual is recorded only once, and (3) all members of the field crew are equally likely to observe reptiles. When these assumptions are met, the rate at which the species composition at a site can be documented using VESs is greater than that rate when using methods that also document species abundances (Doan 2003; Donnelly et al. 2004). Unfortunately, these assumptions are unlikely to be met in most field situations (e.g., the probability of finding an individual organism under a downed log changes among habitats depending on the number of logs available for colonization [Corn and Bury 1990], which violates the first assumption). Because of this, the numbers of organisms observed during VES studies are not necessarily related to the true abundances of organisms in each habitat. Therefore, VES data should not be analyzed statistically unless the assumptions described above have been tested and met at the study site. If these assumptions can be met, then VES methods can be used with great success to monitor populations (e.g., Reagan 1992). In addition, if several observers replicate a VES, then patch-occupancy models can be generated to examine habitat selection of individual species (e.g. Tyre et al. 2003).

Quadrat Sampling

Harold F. Heatwole

Quadrat sampling involves the intensive search of plots (*quadrats*) for reptiles as a means of estimating their biodiversity, relative abundances, and population densities. Jaeger and Inger (1994) evaluated the application of this technique for amphibians and emphasized that quadrats should be placed randomly and in sufficient number to permit adequate statistical analysis, and that they should provide independent data, not repeated measures. These conclusions are equally valid for reptiles, and indeed, in many of the studies employing quadrats both taxa have been assessed simultaneously.

Since the appearance of their review, however, new research has revealed several weaknesses in the technique as commonly employed. In this section I present data evaluating specific details of the procedure and recommend ways to improve its use for sampling reptiles.

Target Groups

Quadrat sampling is amenable only for certain groups of reptiles and is applicable only in certain habitats. Examples of reptilian groups that are obviously not suitable for sampling by quadrats include (1) visually oriented, surface-dwelling species that occupy open habitats and that readily flee from humans; (2) large, highly mobile forms that range over such wide areas that even if they could be confined by quadrate boundaries, the size of the quadrat would be prohibitively large; and (3) arboreal species that use the vertical dimension for escape. Jaeger and Inger (1994) included streamside habitats and even aquatic ones (for larvae) as amenable to quadrat sampling of amphibians. Such habitats have few reptiles, and most that are present are large and/or mobile (crocodilians and turtles). Consequently, quadrat sampling for reptiles in aquatic or semi-aquatic habitats would have limited application, and then only for a few specialized forms, such as semi-aquatic, streamside snakes and lizards. Even these are probably more effectively studied by other methods.

The reptiles most effectively sampled by quadrats are small-bodied species living in or on the leaf litter of forest floors. It is this group to which the method has been applied most often in the past.

Background

Studies using the quadrat method for sampling reptiles have been conducted by Brown and Alcala (1961), Sexton et al. (1964), Heatwole and Sexton (1966), Barbault (1967), Lloyd et al. (1968), Scott (1976, 1982b), Inger and Colwell (1977), Inger (1980), Lieberman (1986), Fauth et al. (1989), Gambold and Woinarski (1993), Allison et al. (1998), Rodda, Campbell, and Fritts (2001), Rodda, Perry et al. (2001), Doan and Arizábal (2002), and Heatwole and Stuart (2008) on a variety of species.

Research Design And Field Methods

Quadrat sampling has two main uses. One is to supplement other techniques in obtaining a list of all reptile species in a geographic area or habitat type. If this is the sole objective, then the requirements for number, size, and location of quadrats are less stringent. It is merely necessary to provide a means of systematically searching for secretive species that avoid detection by other methods. When more quantitative information is desired, such as population densities or relative abundances, then additional requirements must be fulfilled.

There are three major assumptions regarding the use of quadrats for quantitative sampling. They are (1) that the quadrats collectively represent the regions, habitats, and populations being studied; (2) that the animals do not leave the quadrat in response to sampling activities, either before or during the actual counts; and (3) that all animals remaining within the quadrat are found during sampling.

The first assumption requires that quadrats be appropriate in size, placed randomly in the study area, and of sufficient number to allow for valid statistical treatment and interpretation. With certain modifications presented below, the treatment of these topics by Jaeger and Inger (1994) for amphibians also suffices for reptiles. They suggested two sizes of quadrats: 25 to 30 each of 1 × 1 m for *point sampling* of individual species that are small and densely distributed, and 50 to 100 quadrats each of 8 × 8 m for *broad sampling* of larger, more dispersed species or for multispecies assemblages.

For point sampling, they suggested that a grid of the habitat be laid out on paper in a series of large squares. In the field, the investigator begins with one of these squares and moves from its center point for a particular distance and in a particular compass line, both determined from a random numbers table. Then he or she drops a 1- × 1-m quadrat-frame on the forest floor and removes all stones, wood, and leaves within the frame and counts all individuals encountered. This procedure is repeated in the same large square until 10 quadrats have been excavated, after which the investigator goes to a new large square and repeats the process, until all large squares have been sampled in this way.

For broad sampling, the location of each 8- × 8-m quadrat is determined using a random numbers table. The quadrat is then laid out using stakes and twine. Litter is then removed from a 30-cm band around the periphery of the plot to facilitate detection of escapees. Then a minimum of one person per side begins removing litter in strips parallel to his or her boundary, working from the periphery toward the center until the entire plot has been covered. All individuals encountered are recorded.

For both types of quadrat, Jaeger and Inger (1994) suggested recording the following supplementary data: location, dates and times sampling began and ended, temperature, relative humidity, vegetation type, slope, percent canopy cover, depth and percent cover of litter, percent herb cover, height and percent cover of shrubs, number and diameter at breast height of trees, size and percent cover of rocks, and number and sizes of logs.

In an early study (Heatwole and Sexton 1966) and in those studies currently in progress by the author, the quadrats were fenced, whereas in some other investigations they were not. An assessment of this variant of the technique and a test of some of the basic assumptions mentioned above follow.

The procedure for fenced quadrats is more complicated and time consuming than that for unfenced ones. A minimum of four workers mark out a 10- × 10-m quadrat using a compass and a metric measuring tape. A narrow trench about 30 cm deep is dug with a spade around the periphery, and woody obstructions are removed with an ax or machete. Then, one edge of a 2-m-wide nylon mosquito net, long enough to go around the entire perimeter, is buried in the trench. About 15 to 20 cm of the edge to be buried is folded over around the edge of the spade and pushed into the trench so that when in place the fold curves upward on the inner side in such a fashion as to trap any animal attempting to escape by burrowing down along the edge of the netting. This is called the *J-fold*. The spade is removed, the trough of the J-fold filled with soil, and the earth tamped into place. Once the edge of the netting has been buried around the entire border of the quadrat, the above-ground part is rolled up and covered by the leaf litter moved aside when digging the trench. This procedure is designed to restore the habitat as much as possible and to remove a visual barrier to animals moving into and out of the plot. No human activity is allowed around the quadrat during the following afternoon and night, allowing animals that may have been frightened by the activities to return to their customary sites. Early the following morning after most nocturnal animals have retired and before most diurnal ones have become active, the leaf litter covering the netting is removed and the netting unrolled and erected as a fence by tying the top to stakes driven into the ground at intervals around the periphery. During all the above procedures, observers scan the edges of the quadrats to detect any escaping animals. Up to this point in the sampling, all activities are performed from the outer periphery, and no one walks inside the plot.

Once the fence is erected, workers enter the quadrat and systematically walk abreast at 1-m intervals from each other back and forth across the plot, scanning the litter, vegetation and tree trunks in the manner of visual transects, until the entire area of the plot has been covered (called the *walkthrough*). Then, all low plants and shrubs are cut or pulled up, examined individually for reptiles, and thrown out of the plot. Next the organic layer of the forest floor, including dead wood, leaf litter, humus, and any mat of rootlets, is excavated down to bare soil. To do this, handfuls of litter and other substrate materials are removed, placed on light-colored cloth and spread out and searched for animals. Dead wood is broken open and thoroughly searched. After a few handfuls of debris are processed, the cloth is carried to the fence and dumped outside. This process is repeated until excavation is complete, with workers beginning at the periphery and progressing toward the center. All animals found are retained in bags, either to be used as voucher specimens (see Chapter 6, “Voucher Specimens,” and Chapter 7, “Preparing Reptiles as Voucher specimens”) or to be released once sampling has been completed. During excavation, the fence is periodically examined for animals attempting escape.

After the initial excavation and sampling, the plot is left fenced and visited twice daily to collect any animals that may have emerged from subterranean hiding places or that escaped detection previously. The fence is removed only after 2 consecutive days in which no reptiles are encountered. As the fence is removed, the J-fold is carefully examined for reptiles.

Fenced quadrats are time consuming to establish and process. The time required to set up four 10-×10-m plots with fences in Wet Evergreen Forest in Laos, excavate the plots, and check them periodically after excavation averaged 46 person-hours per plot (range 26–65). Values for eight plots of the same size in Dry Evergreen Forest, where litter was sparser, averaged 16.5 person-hours (range 12–20).

Effectiveness of Fenced Quadrats

Fenced quadrats are now assessed in terms of their effectiveness and their fulfillment of the basic assumptions of the quadrat method. As an example, I compare previously unpublished data that Bryan Stuart and I collected from fenced and unfenced plots from the same area and time in Wet Evergreen Forest in Laos during the wet season of 1999.

COMPLETENESS OF SURVEY

For an intensive herpetofaunal survey of Laos, we employed a diverse series of methods, including (1) diurnal transects in streams, at the edges of streams, and in the forest at different distances from streams; (2) nocturnal transects in those same locations; (3) general searching of the forest, including scanning the litter, tree trunks, shrubs and herbs and looking under bark and turning stones and logs and breaking open logs; (4) unfenced quadrats; and (5) fenced quadrats (no attempt was made to sample the herpetofauna of the forest canopy, and thus complete regional representation was not achieved). At any one time, from four to six persons assisted us in these activities.

Even with a relatively small number of quadrats, almost a third of the species of reptiles obtained by the survey was found only in fenced quadrats and by no other method. The species found exclusively in fenced plots included 25 percent of the undescribed taxa for the survey and 43 percent of the new records for the country. Clearly, without the use of fenced plots the survey would have been incomplete and would have missed some of the taxonomically and biogeographically most interesting species.

Other methods, especially streamside transects, yielded species not found in quadrats. Thus, quadrats alone are not completely representative of a region—part of assumption 1, (see “(1)” under “Research Design and Field Methods,” above)—but they are an essential supplement to other methods if the aim is to maximize regional coverage.

ESCAPE OF ANIMALS UPON INITIAL APPROACH

The initial approach to a site by humans undoubtedly frightens some reptiles from the area selected for the quadrat. Observers watching for just such events caught one individual fleeing a quadrat before fencing was completed. It was a surface-dwelling, mobile snake and represented only 1.2 percent of the total of 82 individual reptiles collected in the four plots (Table 18). One would not expect the number of individuals attempting escape during the initial approach to be altered by whether the plot would subsequently be fenced or not, and the two methods would not seem to differ on this point.

Quadrat size would seem to be a factor. Since escapes occur across the peripheral boundary and the number of animals present is determined by the area of the plot, the proportion of individuals lost through initial escape should be lower for larger quadrats (larger quadrats have a relatively smaller periphery-to-area ratio than do smaller ones). This is one of the reasons why small quadrats are not suitable for large, mobile species, but need to be restricted to small, secretive ones. I recommend that 8 × 8 m be the *minimum* size for broad sampling by fenced quadrats; larger ones would be even better.

There are no quantitative data from either fenced or unfenced quadrats as to how many animals leave the plot by fleeing beneath the litter during the initial activity at the periphery. The area disturbed is about the same in both cases as the buffer zone stripped of litter around unfenced plots is about 30 cm wide, approximately the same width as the area denuded for placement of a fence. Activity is more prolonged at the periphery during fencing than when merely excavating litter from a buffer area. On the other hand, allowing an afternoon and night for animals to return to their previous locations before erecting the fence may at least partly offset the initial disturbance. Although there are no data on whether fenced or unfenced quadrats are the best in terms of preventing escape of peripheral animals under the litter, it does not seem likely that differences would be great.

TABLE 18

Numbers of Individual Reptiles Recorded in Fenced Plots in Wet Evergreen Forest[a]

Quadrat	*Number of Individuals Found During* First Approach	Walkthrough	Excavation	*Number of Individuals That Would Have Escaped*	*Total*
A	0	0	12 (4)[b]	7	19 (4)
B	0	0	17 (5)	9	26 (5)
C	1	0	18 (0)	5	24 (0)
D	0	0	13 (1)	0	13 (1)
Total	1	0	60 (10)	21	82 (10)
% of Total	1.2	0	73.2	25.6	100 (100)

a. Bolaven Plateau, Laos, Wet Season 1999.

b. Values for reptile eggs in parentheses.

ESCAPE OF ANIMALS DURING EXCAVATION

Fencing does make a difference in the numbers of individuals found during excavation of the litter. One of the assumptions of the method is that all animals are found during excavation (see "(3)" under "Research Design and Field Methods," above), and the relative usefulness of fenced and unfenced quadrats is determined by how closely they approach that ideal.

We determined that in fenced plots, any animals found climbing the fence, jumping against it, or attempting to dig under it while excavation was in progress had already evaded the excavators and would have escaped unnoticed had it not been for the fence restraining them.

Similarly, animals that appeared in fenced quadrats on days following excavation of the litter would have been missed in unfenced quadrats. It is possible that some of these enter the plot by climbing the fence from outside, although none of the abundant species seemed adept at climbing the netting. Some animals were known to have successfully secreted themselves (perhaps underground), even in plots excavated to bare soil. Two lizards autotomized their tails during attempts to capture them. The tailless animals were not found subsequently during excavation but did appear in the plot on a later day; they were identified by matching them with their shed tails.

Finally, at the end of the sampling when the fence was removed, animals caught in the J-fold would have escaped undetected had it not been for the fence. Thus, animals caught on or against the fence during excavation, found in the plot on subsequent days, or trapped in the J-fold at the end of the sampling period collectively indicate the number of individuals that would have escaped undetected from the quadrat had it not been fenced. This constitutes a comparison of fencing versus not fencing, with all other variables remaining equal. In the example from Laotian Wet Evergreen Forest, slightly over a quarter of the individuals would have escaped detection were it not for the fence (Table 18). Thus, unfenced quadrats seem to allow a significant number of animals to escape during excavation (falls short of fulfilling assumption 2; see "(2)" under "Research Design and Field Methods," above) and can seriously underestimate population densities, thus falling short of fulfilling at least part of assumption 1 (see "(1)" under "Research Design and Field Methods," above). Lloyd et al. (1968) estimated that 5.6 percent of the individuals of reptiles and amphibians escaped from their unfenced plots, although some of these were seen and tallied as they escaped.

Species richness may not be affected so much, as most of the animals that would have escaped from the Laotian plots belonged to the more common species. In the present example, species richness would have been underestimated by only one species, because all other species that occurred in the "would have escaped" category were otherwise found during excavation.

EFFECT OF LITTER REMOVAL

It is clear from the above section that yields from unfenced plots are lower than those from fenced ones of equivalent size. A further procedural dichotomy is whether or not the litter is removed entirely from the quadrat during sampling, or merely removed from its original location without taking it out of the quadrat. The literature is not always clear on which of these procedures was followed. Six quadrats (each 10 × 10 m), in a block design with 10 m separating the plots from each other were used to compare yields from different treatments. Three quadrats were fenced as discussed above; two quadrats were unfenced and excavated by the same procedures, except that each worker, instead of removing the litter entirely from the plot, examined it and then placed it behind him, but leaving an intervening bare zone, as he proceeded from the periphery toward the center. The sixth plot was unfenced and the litter excavated as for other unfenced plots; no reptiles were found, although some frogs were. Then it was fenced and the litter reexamined and removed from the plot (13 reptiles of three species were found). The former data are included in results for unfenced plots in Table 19 and the latter are included in those for fenced plots.

On average, the unfenced plots in which litter was removed from its original site but not removed from the plot yielded only about a tenth as many individual reptiles and about a fifth as many species as those that were fenced and litter thrown over the fence (Table 19). The difference between the two groups is significant for individuals (Mann Whitney *U*-test; $P = 0.028$) and near the border of significance for species

TABLE 19

Yields of Reptiles from Fenced and Unfenced Quadrats at a Single Site[a]

Method/Plot	*Mean Individuals/ Quadrat*	*Mean Species/ Quadrat*
Unfenced: litter not removed	2.0	0.7
Fenced: litter removed	20.5	3.8

[a] Wet Evergreen Forest, Bolaven Plateau, Laos, Wet Season 1999.

($P = 0.057$). Clearly, failure to completely remove litter from plots falls short of fulfilling assumption 3 and renders the method inadequate. This is probably because reptiles can, unobserved by the investigator, gain refuge in nearby litter that has already been examined. Most herpetologists have been amazed at how effectively small lizards can "disappear before one's very eyes" and not be found again. Fencing plots and removing litter facilitates finding such animals later.

Rocha et al. (2000) found that the yield of frogs from fenced plots from which the litter was not removed was greater at night than during the day. They attributed this result to the enhanced visibility of frogs at night because of their eyeshine and their greater activity then; this implies that a number of individuals were avoiding detection by day. The same might apply to nocturnal reptiles such as geckos, and the reverse to diurnal reptiles. The method I recommend here circumvents this diel effect. Removing the litter from fenced plots facilitates the immediate detection of animals regardless of activity level or overt visibility. Likewise, by leaving intact fences around bare plots for several days after removal of the litter, investigators eventually find initial escapees, both nocturnal and diurnal. For similar reasons, variation in yield arising from day-to-day, weather-related differences in activity levels is avoided. Seasonal differences that involve sustained inactivity in refugia below ground would still remain as at certain times of year some animals would be inaccessible, even after complete removal of ground cover.

A further indication of the efficacy of removing litter from fenced plots is provided by Rocha et al. (2001). Although they studied frogs, their results are relevant to the present study. They found that small plots (2 × 1 m) yielded density estimates six times higher than those from large plots (8 × 8 m), contrary to expectation. However, they did not remove the debris from their large plots but did from their small ones. From the data above on the effect of litter removal, it seems likely that their relatively lower yield from the larger plots was not because of size per se, but because many individuals escaped unseen into the litter that had already been searched but not removed. It is likely that both methods underestimated the numbers of individuals, the small ones because of the edge effect noted above, and the larger ones because litter was retained, the error being greater for the larger plots.

COMPARISON WITH VISUAL SCANS

Some transect methods rely upon systematic visual searching for reptiles along predetermined paths or for set periods of time. This method is inadequate for litter reptiles. Initial searches of the plots, mimicking that technique (Table 18), did not reveal a single individual reptile in the present example (although some frogs were found in this way).

In summary, there are certain characteristics that make fenced plots more effective than unfenced ones for the study of forest-floor reptiles. One is the fence itself, which impedes the escape of individuals that would otherwise escape unnoticed. Another important feature is the J-fold. It traps would-be escapees that attempt to burrow beneath the netting; merely burying the straight edge of the netting would not, contributing to an underestimate of the number of animals present. Finally, fencing permits examination of the quadrat over an extended period and the detection of animals that temporarily retreat to underground refuges. Removal of the litter from the plot is essential for obtaining accurate results. Visual scanning of the litter is ineffective for quantitative study of litter reptiles.

Resources

The materials required for quadrat sampling are (1) a metric tape at least 40 m long, so as to reach around the periphery of a 10- × 10-m plot; (2) flags for marking corner posts; (3) stout spades for digging trenches; (4) axes or machetes for cutting woody obstructions in the trenches, cutting down saplings, and cutting stakes for supporting the net; (5) several nylon mosquito nets, each 2-m wide and at least 40-m long, for fencing quadrats (longer ones are even better because they allow overlap around the corner post and provide extra material for repair of holes); (6) string to tie the top of the net to supporting posts; (7) squares of strong, light-colored cloth to serve as a background for searching litter and for transporting it out of the plot; (8) cloth bags for holding animals; (9) field notebooks; (10) metric ruler for measuring depth of leaf litter; (11) compass; (12) environmental monitoring equipment such as thermometers and psychrometers; and (13) fixing trays and tools, tags, and anesthetics and preservatives, if vouchers are to be prepared. Sufficient personnel should be recruited to dig the trench and erect the fence in a timely fashion. One person working alone cannot effectively conduct this work and at the same time scan the periphery for escapees. Also, quadrats that take up to 30 person-hours or more for excavation alone (as some do) cannot be excavated in a reasonable time by one or a few individuals.

Data Treatment and Interpretation

Excavated quadrats constitute destructive sampling. Even if the litter is replaced and the captured animals released, the habitat has been altered severely, and it is unlikely that resampling the same quadrat at a later date would provide meaningful results. Consequently, when an area is sampled over time, old quadrats must be eliminated from the permissible sites for subsequent samples.

Quadrats are labor intensive and time consuming, but they yield species not usually found otherwise and provide quantitative information on both absolute and relative densities. Fenced quadrats require a greater effort than unfenced ones but provide a greater and more representative yield. An investigator must weigh the relative importance of these advantages and disadvantages. If fenced plots are used, it may not

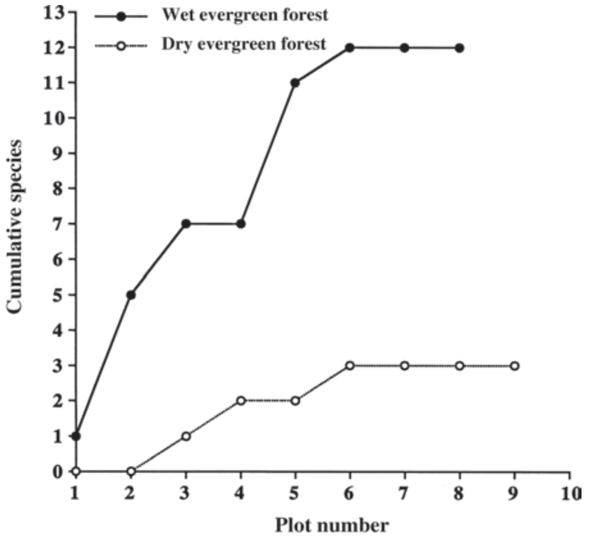


FIGURE 73 Cumulative number of species of forest-floor reptiles encountered in successive 10- × 10-m quadrats in two tropical forests in Laos. Note that dry evergreen forest has fewer species than wet evergreen forest. In both forests, the number of species seems to have reached an asymptote, suggesting that most of the species have been found.

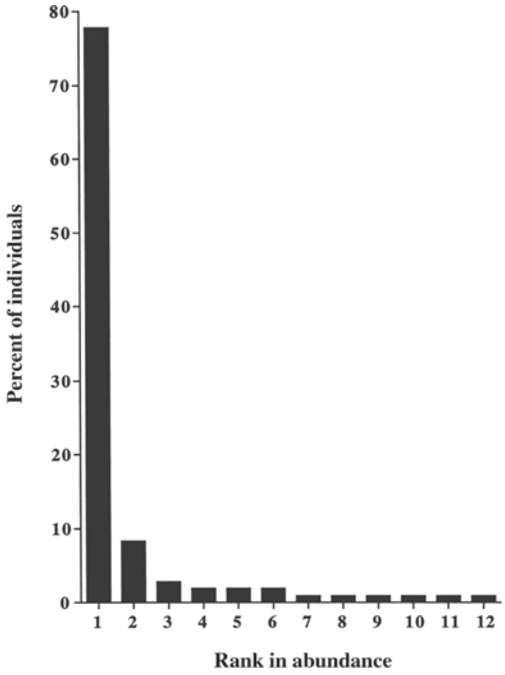


FIGURE 74 Equitability of the forest-floor reptiles taken in eight 10- × 10-m quadrats in wet evergreen forest in Laos. Note that the first-ranked species is exceedingly abundant, whereas most of the species at other ranks are uncommon or rare.

be feasible to have as many replicates as with less demanding methods. On the other hand, the greater accuracy of fenced plots may mean that fewer of them are required than would be the case for unfenced plots. Certainly, with only a few large quadrats one can determine which species occur in great abundance and assemble a considerable list of rarer ones. The actual number of replicates required for quantitative assessment may vary from one region or habitat to another. A reasonable way of assessing the number of quadrats required for a particular area is to construct a species accumulation curve, plotting the number of new species added against the number of plots excavated (Doan and Arizábal 2002). When the number of species reaches an asymptote (Fig. 73), it is likely that all but the rarest species have been found, and unless the numbers of individuals of the commonest species are highly variable, it is also likely that the densities of those species have been reasonably estimated. A recommended criterion is to continue sampling until at least some predetermined minimum number of quadrats have been excavated after the sampling unit under comparison (be it a season, habitat, or geographic region) has reached its asymptote. If that ideal cannot be achieved, the Michaelis-Menten equation can be used on species accumulation curves to extrapolate to total species richness (Hofer and Bersier 2001). Statistical treatment is more effective if the numbers of quadrats for all sampling units are the same.

From quadrat data one can ascertain species richness, absolute and relative densities of component species, equitability, and species diversity. *Species richness* is simply the number of species present. *Population density* is the number of individuals of a particular species per unit area, and *relative density* indicates the abundance of one species in proportion to others. *Equitability* is a measure of assemblage structure in that it indicates how the total number of individuals is apportioned among species; this can be portrayed either graphically (Fig. 74) or as an index. *Species diversity* combines species richness and equitability in a common index. If both species richness and equitability are known already, the index adds little of value. If only the index is provided one cannot discern the relative contributions of the two component attributes, and little useful information is imparted (Hurlbert 1971; DeBenedictus 1973; Peet 1975; Heatwole 1982). However, species diversity is used for some purposes and can be extracted from quadrat data. If sampling is conducted in the same general area over time, temporal changes in all these attributes can be tracked. If sampling is conducted at the same times in different localities or habitats, spatial variation in these attributes can be evaluated, and different *econes* (ecologically different morphs, life-history stages, or sexes within a species) identified (see Heatwole 1989).

In addition to the above information, the numbers of individuals of the more common species may be sufficiently great to glean important demographic information and insights into natural history, especially if sampling is conducted throughout a year. Few other methods are so effective in sampling reptilian eggs and young as is the quadrat method (Heatwole and Sexton 1966). The proportion of males, females, and young can provide information on sex ratio and population structure, as well as reproductive cycles. For example, numerous eggs and hatchlings of lizards were obtained during excavation of the quadrats in Laos (Table 20), but none was found by any other method. If animals are preserved, they can be used for taxonomic purposes, and they can be dissected to ascertain stomach contents and the state of the gonads.

Conclusions

To census small, forest-floor reptiles effectively, (1) quadrats should be large and fenced with an inward-facing J-fold; (2) the leaf litter and other organic debris should be examined and removed from the plot; and (3) the fence should be left in place for several days following excavation. Elimination of any of these procedures reduces the accuracy of the results; indeed, results from small unfenced plots are so inaccurate as to be of little value.

Permanent Plots with Mark-Recapture

Craig Guyer and Maureen A. Donnelly

For studies that require long-term monitoring of key populations, establishment of permanent plots remains a principal tool used by reptile biologists. *Plots* typically are mapped areas of land that allow for identification of the locations of particular individuals of one or several species over time. In our definition of the term plot, we include other repeatedly sampled sites such as snake dens (e.g., Diller and Wallace 2002), ponds or similar wetlands (e.g., Plummer 1997), and linear habitats (e.g., Ramírez-Bautista and Benabib 2001). The key feature uniting studies at such sites is that the plots are visited repeatedly at set intervals so that members of the target species can be captured, marked, measured, released, and recaptured. If visits are repeated over long periods, population trends can be described and attempts made to correlate them with environmental or other factors that are thought to affect the biology of the target species. The population data frequently are used as a preliminary (or often, primary) source of information for development of management strategies (e.g., Diemer 1992). If the target species is chosen with care—for example, if it is an umbrella or keystone species (see “Target Species,” in “Overview,” in Chapter 3)—then it may be possible to extrapolate the information to other species in order to develop management strategies for an entire local herpetofauna. A variety of data can be gathered from permanent plots, including data on individuals (e.g., home range sizes, patterns of interaction among individuals). We have restricted our review to those studies intended to document population trends.

Plot sampling is exceedingly labor intensive. For that reason, the number of plots established in any one study is typically small and most often is set by the amount of time and other resources available to the researcher. Therefore, most studies involve a single plot monitored by a single researcher who is interested in a single species (e.g., Galán 1999). Obviously, this limits the degree of confidence with which one can project beyond the bounds of the study site. Nevertheless, the objective of studies using permanent plots frequently is to gather data that can be used to describe population trends throughout a larger area. For this reason, investigators should create as many plots as can be monitored. Data collected can then be explored to determine the degree to which population trends differ among sites within habitats (e.g., Andrews and Stamps 1994) as well as among habitat types (e.g., Kazmaier et al. 2001; Whitfield et al. 2007). Consistent trends among sites are likely to signal global changes that may be of broad conservation concern. Preliminary examination of variation among sites can be used to estimate the minimum number of plots needed to capture the variance among sites adequately (Elzinga 2001). The number of plots required, as determined by a power analysis, is likely to far exceed the number of plots that an average investigator can maintain and likely will require outside resources and logistical support. Nevertheless, in most situations, plots should be replicated as much as is feasible.

Because plot number is generally small, plots typically are located where an investigator has seen many individuals of the target species. In most cases, this will be where population density is greatest, but in rare cases it may only be where visibility of the target species is greatest (Mackenzie and Kendall 2002). In either case, plots are positioned to maximize the rate at which data can be collected. For species for which habitat requirements are known and for which sufficient time and/or personnel are available to allow creation of multiple plots, GIS technology provides a convenient mechanism for positioning plots in a fashion that adequately represents habitat availability for the target species (e.g. Hermann et al. 2002; see also “Using Geographical Information Systems to Design Reptile Surveys,” in Chapter 3). Habitats used by the target species can either be generated from preliminary surveys or from descriptions in field guides or other literature, if available.

Plot size depends upon the size and/or mobility of individuals of the target species. In general, because accumulation of population data that can be used to determine trends is the goal of studies involving plots, each plot should be of sufficient size to include enough individuals to constitute a viable population. The minimum size for viable populations of reptiles is virtually unstudied; therefore, we recommend that plots be large enough to incorporate at least 50 individuals, the minimum number suggested by some computer simulations to constitute a viable population of vertebrates (Franklin 1980). However, accumulating data from vertebrates suggest that typical values for population size may range from 100 to 300 (Smallwood 2001; McCoy and Mushinsky 2007).

Because large-bodied species tend to have lower population densities than small-bodied species, and large individuals tend to have greater mobility (Brown 1995), studies of large reptiles require larger plots than studies of small reptiles. However, the average detection distance for each individual of a large species is greater than that for a small species (e.g., Kazmaier et al. 2001). For cases in which individuals are captured at sites where the same individuals aggregate consistently year after year (e.g., snake dens, Larsen and Gregory 1989; turtle nest sites, Valenzuela 2001), the actual area used by all individuals during a year, and therefore the effective plot size, is unlikely to be known. In such situations, the investigator must interpret the data with great care.

Sampling frequency is a key consideration for studies involving permanent plots. The temptation in such studies is to sample as frequently as possible in order to document trends on the smallest possible time scale. However, the frequency with which any one plot is sampled affects the total number and size of plots that an investigator can maintain. Additionally, the more frequently a plot is visited, the more likely the observations will not be statistically independent, which may affect analyses of those data (White and Garrott 1990). Investigators who intend to use population trends as a basis for designing or implementing management or conservation strategies that can be used at multiple sites should sacrifice sampling frequency in favor of maximizing the number of plots included. For target species with long generation times (>5 years) and a restricted reproductive season each year, plots may require only a single annual sample; therefore, it may be feasible to maintain many plots. For target species with short generation times (<3 years) or multiple reproductive periods each year, plots will need to be sampled more frequently (at least monthly), making sampling of replicate plots more time intensive.

If possible, an investigator should attempt to census all individuals of a target species each time he or she visits a permanent plot. However, because many individuals will be hidden from view, it is more likely that only a sample of individuals will be taken. Regardless, the investigator should attempt to record all active individuals at each visit. Species that can be discovered with a careful survey of the entire surface area of the plot or that can be readily trapped are the easiest to work with. A reptile census may be possible in habitats that are structurally simple, but species occupying structurally complex habitats typically must be sampled. Animals detected electronically via continuous radio signals (e.g., Eubanks et al. 2002) or activated tags (e.g., Webb and Shine 1998b) can be monitored, provided that all individuals using the plot are fitted with transmitting devices. In situations in which censuses are impossible, samples taken of active individuals must be representative of the residents of the plot.

Regardless of whether a census or a sample is taken, individual reptiles at each capture interval are recorded as marked (and, therefore, recaptured) or unmarked (and, therefore, newly captured). Newly captured animals must be given a permanent mark, either as a cohort member (same mark given to all individuals from a particular sample period) or as an individual (unique mark for each animal). An underlying assumption is that individuals do not lose their marks and that they are correctly identified when recaptured. At each capture period, information on size, age, sex, and reproductive status must be taken in order to maximize the utility of the data (see Chapter 10, “Determining Age, Sex, and Reproductive Condition”).

The overall goal of monitoring projects associated with permanent plots is to determine population growth rate of the target species. The demographic variables required to calculate this parameter are age- or size-specific survival and fecundity. Most such analyses focus on females and summarize the data into the form of a life table in which the probabilities of surviving to and reproducing within certain age or size classes is averaged from individuals marked and recaptured over time. Matrix algebra can then be used to determine population growth rate as well as to model the effects of changes in the key population parameters (survival and fecundity) on population growth (Caswell 2001; see Chapter 15, “Population Size and Demographics”). The calculated population growth rate of the target species can be used to evaluate its status. A decreasing growth rate would indicate that some management program may be required to prevent extirpation or extinction. In such cases, the modeling process can be used to determine which segment of the population is most sensitive to environmental changes and, therefore, is most likely to be responsible for the population decline. Armed with these results, conservation efforts can be tailored to maximize the rate at which declining populations can recover. Congdon et al. (1994) modeled demographic parameters of *Chelydra serpentina* based on a 17-y mark-recapture study at a single pond in Michigan. They concluded that successful conservation of this and similar long-lived vertebrates would require special protection of reproductive adults and older juveniles and that protection of nests or production of *headstart hatchlings* (animals reared in a laboratory setting and released into the wild after reaching a size less vulnerable to predation) would likely have little impact on population viability. This kind of interaction in which data carefully accumulated from permanent plots is modeled analytically holds the greatest promise for providing informed management decisions for maintaining reptile faunas.

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Transect Surveys, Including Line Distance

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Transect surveys have been used for decades by herpetologists as a means of standardizing survey effort. In general, this type of survey is designed to detect species through time and space using either a single or repeated survey line or set of points (Krebs 1989). This method can be used to monitor short- and long-term changes in species diversity or abundance, and data acquired can be used to compile species lists and to track abundance, distribution, and relative densities of target species through time and space. Transect surveys are widely applicable across species and habitats and have become a standard method of choice for biologists who monitor populations. Several excellent reviews of line-transect-survey techniques are available in the literature (Anderson et al. 1979; Gates 1979; Burnham et al. 1980; Burnham and Anderson 1984; and Buckland et al. 1993, 2001).

Many federal, state, local, and nongovernment agencies require that rare and/or listed species be surveyed periodically to evaluate population trends. Protocols may require repeated surveys, or only a single assessment to determine presence or absence. Transect surveys vary in intensity. They can be used to survey an area completely or, if the transects are spaced randomly, to sample a subset of an area or habitat. The latter method is usually more cost effective, and when designed properly, statistical inferences can be drawn from the data to estimate the abundance and density of a species with some confidence. Because different methods have different efficiencies, transect surveys can also be used in conjunction with other sampling methods to provide a more comprehensive species list.

Classical Transects

Two categories of transect surveys are recognized: classical transects and distance transects. With *classical transects,* several transect lines or circular plots are placed randomly in appropriate habitat to survey for a particular target organism. One or more observers proceeds along the transect line or remains stationary at the center of the circular plot and records all individuals detected within a predetermined distance from the transect line or central point. Generally, distances between the observed individuals and the transect line or central point are not measured, unless necessary to determine if an individual is on or off the transect. Classical transects are based on the assumption that all individuals within the sample area are encountered and counted. That assumption is rarely tested, and in most cases it is probably violated because of the inherent difficulty of observing every individual. As a result, classical transects in most circumstances underestimate the density of the targeted species. Consequently, despite the considerable effort and expense, mark-recapture studies are often employed to provide more accurate estimates of the sizes of populations.

Distance Transects

Distance transects are designed to minimize the error inherent in classical transects. Typically, one researcher moves along the center of the transect or remains stationary at the center of the circular plot to observe the targeted species while another researcher measures the perpendicular distance from the observed target individual to the transect line or to the center of the circular plot. Additional observers can be used to locate or flush target species from their original locations. Distance transects have gained favor for sampling certain reptile species (Buckland et al. 1993) because they are superior to classical transect sampling in several ways. They are the primary focus of our treatment of this section.

Detection Distance

We begin our discussion of transect sampling with a simple example. Suppose an investigator wants to survey giant tortoises on a desert grassland plain on a remote island. Because these giant tortoises evolved in the absence of mammalian predators, they neither flee nor approach when the observer appears. They do not hide in underground burrows, and neither large rocks nor vegetation is present to conceal them. They are solitary, except when mating, and mating season is months away. Furthermore, the tortoises are distributed randomly across the landscape such that, on average, there are as many individuals in any one arbitrarily large area as in any other. To simplify matters, let us further suppose that a more-or-less straight trail cuts randomly through the 100-km^2 habitat occupied by the giant tortoises, but that the tortoises neither cluster around nor avoid the trail. The challenge is to determine their abundance.

Given the extraordinary visibility of giant tortoises, it seems safe to assume that every individual within some distance of the trail, say, 20 m, is visible. The observer walks the length of the trail and records all tortoises seen within the 20-m-wide transect on either side of the trail (i.e., carries out a fixed-width, classical strip transect). Along the 10-km length of the trail, the observer would survey 40 m × 10,000 m, or 400,000 m^2 = 40 ha. Suppose that the observer sees 37 giant tortoises in those 40 ha. The average absolute population density detected would be 37/40 ha, or 0.925 tortoises/ha. If all of the conditions that were stated previously were valid, then the density estimate should be reasonably unbiased; however, it may not be precise. This method is based on the assumption that 100 percent of the individuals within the fixed-width (40 m × 10 km) transect are detected. However, the distance from the center line of the transect to any giant tortoise was measured only when an animal was near the 20-m limit, to determine whether it was on or off the transect.

While surveying the giant tortoises, the investigator noticed many tortoises at distances greater than 20 m from the trail, and by the end of sampling was certain that she was able to see all giant tortoises within 40 m of trail. To obtain a more precise record of tortoise density she decides to resample, only this time she records all individuals within 40 m of the trail (total area = 80 ha). On this transect she observes 63 tortoises, which computes to an average density of 63/80 ha, or 0.788 tortoises/ha—lower than the first estimate. This causes her to fret about her assumption that she saw every tortoise within 40 m of the trail. Perhaps 30 m would have been more reasonable. Perhaps she should resample tortoises along the trail using the 30-m cutoff.

Exhausted from the 20 km of transects, she decides to reevaluate her sampling design. She now questions whether it is safe to assume that she observed all of the tortoises within 20 m of the trail. Perhaps a small individual was hidden from view behind one of the larger tortoises. She resolves to improve her transect sampling protocol by recording not only the number of individuals observed but also the distance from the center of the transect to each individual. From the arrangement of detection distances she can determine if her giant tortoise–sighting rate begins to decline at 5 m, 10 m, 15 m, or some greater distance from the transect line. In that case, there is no need to decide on a detection threshold a priori; the data will answer that question! The analyses can be done on the computer using the program DISTANCE (Laake et al. 1993). DISTANCE not only fits a curve to the detection-distance results but also uses the function to estimate the absolute population density. Distance sampling (Buckland et al. 1993) is so named because the distances rather than the number of individuals are sampled (although each distance value implicitly denotes the presence of one individual). Distance sampling has largely replaced the use of fixed-width transects because it is a more general and flexible application of the same process. Under the right set of circumstances, distance sampling is a powerful tool for estimating the absolute abundances of animals, and many herpetologists advocate its use for enumerating reptiles in a population (Akin 1998; Thompson et al. 1998; but see Smolensky and Fitzgerald 2010).

Three applications of distance sampling exist for reptiles: *point counts, linear transects,* and *trapping webs*. In each application, the detection probability declines with distance from a center point or a transect line. A linear transect was used for the hypothetical search for the giant tortoise, above, but the same giant tortoise population could have been sampled by observing individuals from random points (point counts) within the habitat. A trapping web is conceptually similar to the point count, except that traps rather than observations are used to detect the animals. Traps are set along lines radiating out from a central point, like spokes around the hub of a bicycle wheel. Because the trap lines converge at the center of the array, the likelihood of capture is maximal and assumed to be 100 percent at the center of a trapping web. Thus, like our hypothetical giant tortoise researcher who observed all individuals on the center of the line transect, a trapping web is assumed to detect all of the animals near the center.

Assumptions for Distance Sampling

To determine if distance sampling is appropriate for a given system (species and/or ecosystem), one must assess the extent to which the system satisfies the assumptions. As is true for all sampling methods, distance sampling should be used only when all the assumptions can be satisfied or if deviations are understood well enough to allow for appropriate adjustments of estimates. Assumptions for valid use of distance sampling are that:

1. All individuals on/near the centerline or point will be detected.
2. The sampling line or points are placed randomly relative to the distribution of the target species.
3. Individuals are detected at their original locations.
4. Distance measurements are without error, unless using belts (e.g. 0–5 m, 5.1–10 m, etc.).
5. Individuals are counted only once.
6. Individuals behave independently (and therefore are detected independently).

ALL INDIVIDUALS ON/NEAR THE CENTER LINE OR POINT WILL BE DETECTED

When we "observe" a reptile, it may be so visible that we assume the detection probability = 1. The assumption that the probability of detection on/near the center line of the transect must be confirmed empirically. On the few occasions when transect surveys have been made with known numbers of observable target individuals (e.g., Desert Tortoise, *Gopherus agassizii*: Anderson et al. 2001; Texas Horned Lizard, *Phrynosoma cornutum*: Henke 1998), all researchers missed some individuals that were in plain view. In the horned lizard study, the average detection rate was 73 percent; no group of students detected all 10 lizards located in a 1.2-ha patch of open land in Texas. Factors overlooked by eyewitnesses of crimes indicate that human observers, no matter how motivated, miss some objects in plain view.

Most reptiles do not remain stationary or in plain view for extended periods. In our giant tortoise example, individuals did not take refuge underground, and vegetation did not obscure them, as might be the case in a real setting. When approached by a human, most species of reptile seek shelter by diving into water, burrowing into the ground, running away, climbing into high vegetation, and generally avoiding human contact/detection. Virtually any type of vegetation can conceal a reptile, and rocks may conceal a reptile by aiding their crypsis or blocking the observer's view. In a test of DISTANCE sampling in a scrubby tropical forest, most lizards and snakes went undetected (Rodda and Campbell 2002).

Several methods exist for calibrating, or at least estimating, deviations from the 100 percent detection probability for individuals on a center transect line. If all individuals of the target species are detectable when they are active, then one can conduct radiotelemetric or rigorous mark-resighting studies to determine the species' activity patterns and estimate the percentage of the telemetered individuals detected under different sets of conditions (habitat type, time of day, season, recent rainfall, etc.). One may have to compensate for differences in activity patterns based on size, age, body condition, and sex of the animal. For this approach to provide useful data, the factors that influence detection must be known with sufficient accuracy to use them in complex mathematical models without excessively inflating resultant variance estimates (see Buckland et al. 1993 for robust variance estimation procedures).

Another approach to calibration of the detection probability of a given target species is to study visible sign of the targeted species (see "Reptile Sign and Camera Stations," below). For example, one can measure the distance from the transect center line to features such as shells of dead turtles or scat. Because we are most interested in an estimate of the density of the live animals, we must determine the relationship between the density of the object surveyed (shell or scat) and the target species. To understand this relationship, one may need to study decay processes and determine the rate of disappearance of sign (Dodd 1995). For highly durable sign, such as tortoise shells in arid habitats, the shells can be inconspicuously marked to determine if the same shells are found in subsequent surveys (mark-recapture analysis). Unfortunately, sign may be more difficult to detect than living animals. An inaccurate relationship between sign and the living animal may inflate variance estimates to an unacceptable degree (Thompson et al. 1998).

If a detection probability is less than 1, then it is likely that different researchers will have different error rates. Differences among researchers in their ability to detect the target species will inevitably cause the population estimate to be low. If two researchers observe different numbers of individuals, then the greatest percentage that a researcher can be observing is 100 percent of the animals on the center line, and the other is observing less than 100 percent. Because all researchers likely will miss some individuals, a validation study is essential to determine rates of detection.

The assumption specifies that detection probability = 1 on or "near" the center line; but how near is "near"? The correct answer depends on the analytical method used. Typically such data are analyzed by grouping records that fall within "*belts,*" that is, areas between predetermined boundaries that run parallel to the transect. For example, one might group all observations detected within belts lying from 0 to 1.9 m, 2.0 to 3.9 m, 4.0 to 5.9 m, and so forth, from the center line. In this example, "near" is defined by the limit of the first interval, that is, within 2 m of the center line. Confidence limits will be tightest if additional distance intervals lie within the 100 percent detection zone. In the above case it would be highly desirable to obtain 100 percent detection out to 4 m. The choice of detection intervals (in this case 2, 4, and 6 m) can influence the accuracy and bias of distance sample estimates. A substantial amount of literature exists on the merits of different detection intervals (Buckland et al. 1993), but the investigator should experiment with different intervals to determine the extent to which a given data set is sensitive to the interval selection.

SAMPLING LINE OR POINTS ARE PLACED RANDOMLY RELATIVE TO THE LOCATIONS OF ANIMALS

Walking along a road or path usually violates this assumption in uplands, and working along the margin of a body of water or parallel to depth contours is also a violation. In densely vegetated areas, however, a road, path, or the water's edge may be the only reasonable option. Microhabitats near roads often differ from those far from roads. Water infiltration may be reduced on a road, leading to greater moisture availability at the road edge. Weedy species of plants often invade road or trail edges. Sunlight generally penetrates to the ground in forested areas fragmented by roads or trails. Such subtle and anomalous edaphic and vegetative features can distort distance samples by altering either the distribution or the visibility of the animals (e.g., heliophilic lizards that come out to a trail to forage or bask; animals concealed by lush vegetation) that are present. To our knowledge, the significance and implications of this assumption have not been assessed for any reptile species. The underlying problem is that in many habitats, transect sampling is not practical except near a road or trail. With few exceptions, such habitats are judged undesirable for distance sampling (Buckland et al. 1993). One suggestion to overcome bias in placement of the sampling line is to sample parallel to any existing clinal or habitat gradient, since this approach will distribute samples among all habitat types.

INDIVIDUALS ARE DETECTED AT THEIR ORIGINAL LOCATIONS

At best, this assumption is reasonable for only a few reptile species (e.g., slow-moving tortoises or alligators in a lake at night). Most lizards, snakes, and basking pond turtles attempt to flee from any human intruder, and often do so, before being detected or identified positively. The assumption that animals are detected at their original locations is subject to the same bias as the assumption that requires 100 percent detection. We see some animals that do not flee, but we do not see the many that remain motionless or those that flee in response to the observers' approaching footsteps. Nocturnal animals that remain motionless when illuminated with a spotlight would appear to satisfy this assumption. To our knowledge, no tests of this assumption have been published for reptiles. We are aware of no alternative method to satisfy this assumption.

The ability to detect individuals at their original locations is of special importance when using a trapping web, as the time interval during which trapping is conducted (hours to days) is much larger than the time interval during which visual scans are conducted (typically a few minutes at a point). If animals congregate near the center of a trapping web, the web will produce an overestimate, and vice versa. For example, small skinks (*Carlia ailanpalai*) have been observed (GHR, pers. observ.) to be attracted to their trapped conspecifics, causing trapping webs to overestimate localized skink abundance. If, on the other hand, human activity associated with the high concentration of traps at the center of a web causes individuals to flee, then abundances will be underestimated.

DISTANCE MEASUREMENTS ARE WITHOUT ERROR

If the width of a transect is chosen in advance of a study (as it should be), then distances need to be measured accurately only for those animals found at or near the margin of the transect. If, for example, the observation intervals are 2, 4, and 6 m, then it is not necessary to precisely measure the distance from the transect center line to a reptile that is about 5 m from the line. It suffices to know that the animal was in the third interval (4-6 m).

A problem develops with the assumption that distances are measured without error if distances are estimated and the estimate is consistently high or low. Unbiased errors in distance estimation have little bearing on the mean density estimate. Such errors may inflate variance estimates, however.

INDIVIDUALS ARE COUNTED ONLY ONCE

This assumption is most likely to be violated if an animal responds to, or is independently moving in the same direction as, an observer. If a fleeing individual comes into contact with another observer or with the same observer later, then it may be counted twice. Double counting would not appear to be a serious problem for continuously visible species, but it could significantly distort counts of wary and intermittently invisible species such as crocodilians, which submerge and reappear elsewhere.

INDIVIDUALS BEHAVE INDEPENDENTLY (AND THEREFORE ARE DETECTED INDEPENDENTLY)

Target species that occur in groups or flocks are most likely to violate this assumption. The solution is to treat each flock as the object to be sampled; an average estimated flock size can then be calculated from observations. Protocols developed for sampling flocks of birds should be consulted for those few reptile species that tend to occur in groups (Buckland et al. 1993).

The information presented thus far is pertinent to distance sampling with the intent to establish the absolute density of the target species. With a few additional assumptions and qualifications, distance sampling also can be used for presence/absence surveys or estimates of the relative density of targeted species.

The problem with presence/absence data is that one can never "prove" an absence. The best one can do is to compile data rigorously, thereby making a case with some estimated probability that an unobserved target species is absent. The crux of the problem for distance sampling is that it is impossible to derive the shape of the detection function when there are no detections. In that case, one assumes that the detection function in the area where the target species was not located is similar to the detection function in an area where it was present; the detection function is then calculated based on those data). Measuring the habitat factors that influence observation of the target species can strengthen the argument by demonstrating that the factors are similar in "present" and "absent" sites. For example, suppose that a multivariate analysis indicates that detection of a target species is largely dependent on the height of the grass. Suppose further that data indicate that the height of the grass was lower in an "absent" area than in a "present" area. That provides evidence that detection of the target species should be nearly the same in both areas. The uncertainty associated with demonstrating "absence" must be estimated from the area where the target species was undetected, the confidence you have that the detection function was the same at present and absent sites, and the confidence limits on detection associated with the site where the species was present. No exact method exists for quantifying confidence that a detection function is the same at two sites, so it will not be possible to provide an absolute quantification of the uncertainty associated with judging a target species to be absent.

Using distance sampling to estimate relative population sizes requires that the factors influencing detectability have the same value for all comparisons. The assumption that the probability of detection on the center line is 1.0 is relaxed, however. Establishing that two functions are the "same" is difficult, as differences can be subtle. Accordingly, the level of precision required to address a specific question must be decided in advance. For example, suppose that one wishes to compare the year-to-year densities of a specific lizard in desert grassland. Suppose further that there is only one observer and that he or she has exhaustively quantified his or her ability to sight this species, and that the ability hinges almost entirely

TABLE 20

Key Assumptions Generally Applied to Classical and Distance Transects

Assumptions	*Classical Sampling*	*Distance Sampling*
(1) All target species in transect area are detected.	X	
(2) All individuals on transect line or point are detected.	X	X
(3) Extent of the entire area sampled is known.	X	
(4) Distances from the transect line or points to observed individuals are accurately measured.		X
(5) Individuals are not disturbed by the observer before detection.	X	X
(6) Individuals are counted only once.	X	X
(7) Individuals are detected independently.	X	X
(8) Bias (from observers, methods, conditions) is understood.	X	X

on the grass cover. Furthermore, the relationship is fairly linear, with detection peaking at 80 percent when no grass cover is present and declining to 45 percent when grass cover is maximal (80% cover is maximal for this hypothetical grassland). In this case the annual changes in grass cover can be modeled, and the extent to which the annual fluctuations in numbers of lizards observed is a result of their changing abundance or the change in grass cover can be determined. Note that this quantitative detectability model also allows for the estimation of absolute abundance of the lizard. In other words, if knowledge about detectability is sufficient for quantitatively modeling the sources of bias in detectability, then it will be sufficient for estimating absolute population density. If, on the other hand, the idea that denser grass equates to fewer lizard sightings is only a vague notion, it will not be possible to determine whether the observed annual changes in lizard sighting rate were caused by changes in grass cover or changes in lizard abundance or the proportion of change caused by each factor independently (both are likely to be involved).

In many circumstances, the use of relative abundance measures amounts to an admission that one is making an untested assumption that detectability is identical under the conditions prevailing in the comparisons of interest. Such assumptions are particularly troublesome when they involve different vegetation conditions (such as interhabitat comparisons, changing seasons, or annual progression of vegetative changes), different observers (in the absence of interobserver skill quantification), or different species (rarely do two species have identical detectabilities). For these reasons, relative abundance measures have limited utility for long-term population monitoring.

Target Species

Not all reptiles lend themselves to sampling using transect methods. Species that are hard to detect, such as fossorial, saxicolous, rupicolous, arboreal, small, or cryptically colored forms are problematic, if not impossible, to sample (but see Chapter 11, “Techniques for Reptiles in Difficult-to-Sample Habitats”), and transects likely will not provide reliable data. For some small, fossorial species, however, sign (e.g., burrows, tracks) may be used as evidence of species presence along transects (Sutton et al. 1999).

Generally, any reptile that can be detected reliably and with reasonable accuracy can be surveyed effectively with transects, although the technique works best with relatively large, conspicuous species. As detectability of a target species decreases (e.g., due to size constraints or habits), however, several of the assumptions of classical and/or distance transect methods become increasingly difficult to satisfy. Classical transect sampling assumes that 100 percent of the target species within a transect area are detected, whereas distance sampling assumes that 100 percent of the target species on the center line of a transect are detected (Table 20). The assumptions of the method and detectability of the target species should be carefully considered prior to beginning a project using the transect technique. The efficiency (minimal cost and use of resources) of obtaining absolute population density using a particular method should be maximized by the study design.

Background

Many investigators have used transect surveys in their studies of reptiles. In this section we provide examples from those studies. Although our coverage is not comprehensive, it should direct investigators toward some excellent studies through which they can familiarize themselves with the technique. Indeed, transect-type techniques are commonly used in a variety of ways to survey for reptiles. Surveys using transects and visual observations have been used to determine distribution and density of the African Dwarf Crocodile (*Osteolaemus tetraspis*; Riley and Huchzermeyer 1999), St. Lucia Whiptail Lizard (*Cnemidophorus vanzoi*; Dickinson et al. 2001), both Bahamian (Knapp 2001) and Fijian iguanas (Harlow and Biciloa 2001), and mixed-species lizard (Romero-Schmidt et al.1994) and snake (Sullivan 2000) populations. Snake populations have also been studied by researchers driving along a highway transect (Rosen and Lowe 1994). Cassey and Ussher (1999) tested line-transect versus mark-recapture techniques to determine tuatara abundance. Transects have been used for species inventories (Nussbaum et al. 1999; Lamb et al. 1998). Aerial transect surveys are widely used in studies of sea turtles (Epperly, Braun, and Chester 1995; McDaniel et al. 2000) and have also been applied to crocodilians (Mourão, Bayliss, et al. 1994). Transects have also been used for surveys of dinosaur remains (Brinkman et al. 1998) and for extensive species inventories along transects more than 100 km long (Lehr et al. 2002).

Tortoises have been used widely in studies of transects as tools for estimating density and abundance. Gardner et al. (1999) studied the use of visual transects to survey the Geometric Tortoise (*Psammobates geometricus*). Anderson et al. (2001) tested the practice of line-transect methods as applied to surveys for the Desert Tortoise (*Gopherus agassizii*). Researchers have used classical transect methods to estimate the relative abundances of the Gopher Tortoise (*Gopherus polyphemus*) in selected sites in Florida (Mushinsky and McCoy 1994; McCoy and Mushinsky 1995). Burrows of the Gopher Tortoise have been used as a surrogate for direct surveys of this species along transects. Three researchers walking parallel to one another about 2 meters apart located and measured each burrow along the transect. Because the widths of burrows correlate strongly with the carapace lengths of the resident tortoises (Martin and Layne 1987; Wilson et al. 1991; McCoy et al. 1993), they were able to construct a demographic profile of Gopher Tortoise populations. These researchers used belt transects (7 m wide) of various lengths to determine the areal extent of the habitat occupied by tortoises rather than a subjective evaluation of habitat suitability (Mushinsky and McCoy 1994).

Research Design

The specific transect method and study design used for any given sampling project should reflect the nature of the research question being asked. Transects can be used to detect the presence or absence of a species, to estimate population size or density, to detect the boundaries of populations, and to detect variation in population size or density through time. Transect surveys ideally are applied to inferential studies, such as those comparing the abundance or density of a target species among different habitats, among different treatments, at different times, or along gradients. Transect surveys are efficient when time and resources for more extensive or expensive studies (e.g., mark-recapture or radiotelemetry) are limited. Before an investigator begins transect surveys, however, she or he should consider several factors, including financial constraints, availability of personnel to conduct the surveys and any training that they may require, assumptions associated with transect surveys to ensure that they are met in the field, and familiarity with data treatment.

Distance models, refined over the last few decades (Buckland et al. 1993), require that the perpendicular distance from the transect line or central point to the location of the target individual when first observed be measured. Unless specifically needed, boundaries of the sample area need not be determined. Use of a site-specific detectability function eliminates the need to assume that all individuals in the sample area are detected. The detection function specifies probabilities of detecting the target species relative to the distance from the transect line or central point. This function makes the estimation of density (with appropriate assumptions) with a greater accuracy than classical transect methods possible. Assumptions of classical and distance models are compared in Table 20. Use of the computer program DISTANCE (see Appendix II) greatly facilitates analysis of data generated by distance sampling (Laake et al. 1993).

Most reptile researchers use line transects for distance sampling, especially in relatively open habitat. Point sampling may be preferable in relatively dense habitat or if linear transects are environmentally constrained. Trapping webs and indirect counts of scats, burrows (Iverson 1979c), or other sign can be regarded as variants of the basic distance theory applications (Buckland et al. 1993). Line transects must be of known length and as straight as reasonably possible. Deviation from absolute "straightness" is acceptable, as long as detectability is not compromised and transects are placed randomly within representative habitats but sufficiently far apart to avoid double counting of individuals. Established routes such as roads and ridge top trails are subject to bias and should be avoided. Clustered populations warrant special considerations (Buckland et al. 1993). Preliminary surveys are particularly important for making decisions on transect method and design. Buckland et al. (1993) have provided an excellent discussion of options to consider before deciding where to place transect lines or points.

Measuring distances can be cumbersome, making distance transects more time consuming than classical transects, particularly if the investigator must leave the transect line to mark the location or measure the distance to the initial location of each target animal. At the same time, the researchers may disturb undetected target individuals, nearby. However, new and relatively inexpensive technologies can greatly expedite data collection, if used with care. Laser rangefinders are relatively inexpensive and can be used to estimate distances from the transect line to the observed individual. Also, researchers can estimate the sighting angle, relative to the transect line, by use of a hand held angle board or an angle plate on a tripod (Buckland et al. 1993). By rapidly collecting and recording distance and angle measurements for each individual observed, perpendicular distances can be calculated using common trigonometric functions. Less technologically advanced methods, such as counting paces and visual distance estimation, can be used if the concomitant loss of accuracy is acceptable. Note that accurate measurement of the distance to a target individual that is near the center line of the transect is critical because of the mathematical properties of distance models. When distance estimates are less accurate, investigators commonly truncate the observations (remove the outliers) before the data are analyzed by setting a distance limit; they also round distances to convenient values and collapse data into distance categories. Observations should not be truncated to an extent that introduces systematic bias against outliers within the data set.

Distance-sampling models assume that a target animal does not move in response to disturbance by the investigator prior to detection and that individuals are detected independently. These assumptions may be problematic if densities of target species are high or if individuals are readily detected. The potential exists for double counting, especially if individuals move rapidly in response to the presence of the researcher. In some circumstances, it may be advantageous to leave the transect line to search and probe for target individuals. Ideally, several observers walk on each side of the center line of the transect, communicating their sightings (with hand signals, if voices disturb the target species) to the one recording the data (Mushinsky and McCoy 1994).

For comparative and experimental studies, standardization and repeatability of sampling methods, efforts, and conditions during replicate surveys are important. Most critical is the assumption that 100 percent of the target species on the center line are detected. Repeatability is enhanced if the start and end points of each transect are determined with a Global Position System (GPS) unit.

Field Methods

A researcher must first define the goals of the transect survey. Exactly what question(s) are to be answered with the transect data? The target species must be detectable directly or by other obvious sign if a transect survey method is to be used. Ideally the technique is applied to single-species surveys. Henke (1998) showed that searching for multiple objects has a very detrimental effect on detection, with the largest decrement occurring when searching for more than two species. Prior knowledge of the general distribution, habitat preferences, and behavior of the target species will improve the quality and validity of the data collected. The more background information available for the target species, the better the opportunity to collect meaningful data. Knowing the type of habitat to survey, the appropriate place to search within that habitat, and the most productive season of the year and time of the day to conduct a survey increases the likelihood of a meaningful study. With sufficient prior knowledge, a researcher can construct a data sheet to standardize data collection. Novice researchers should consider surveying several trial transects to identify the best techniques and to refine the data sheet.

The transect methods and design must provide an adequate sample size. As a rule, the minimum sample size from all transects combined should be 60 to 80 individuals, or “detections,” of the target species, although 40 may still provide reasonable precision (Buckland et al. 1993). Somewhat larger samples (25% more) are recommended for point sampling and for sampling clustered populations. In contrast to mark-recapture studies, the absolute size of the sample, rather than the fraction of the population sampled, is important for distance sampling. For studies of rare or endangered animals, such sample sizes may be unachievable, and alternative methods of analysis may prove more informative. A detailed discussion of appropriate sampling design and procedures is presented in Buckland et al. (1993).

Resources Required

Ideally, two or more persons should execute each transect, for several reasons: (1) safety, which in remote areas is essential; (2) division of labor (one person records all data); and (3) to ensure the quality of the data collected, which is a reflection of the effort made. Teams of researchers learn to work together, and their efficiency improves with experience. Using two persons can be costly, both temporally and financially, and in some cases a single person may be forced to conduct the transect survey and record the data. In some circumstances, sampling by a single individual may be an advantage because the presence of an additional person may increase habitat disturbance and/or affect the natural movements of the target species.

Equipment used in the field reflects the nature of the study and the rigors of the habitat. Essential supplies are pens with permanent ink or pencils and data sheets on a clipboard. Additional equipment may include a compass, a handheld computer for data entry (see “Handheld Computers for Digital Data Collection,” in Chapter 4), a rangefinder to determine distances, a handheld Global Positioning System unit for recording transect locations, and a plastic bag or other waterproof container for the data in case of rain.

The time required for a transect survey depends upon the target species and its abundance, the area to be surveyed, and the goals of the study. Relatively long transects may be needed to traverse large areas. If researchers need to walk slowly because the target species is responsive to their presence, then the distance covered per unit time may be relatively low. Habitat constraints may require numerous relatively short transects, and the amount of time spent traveling between transects can add substantially to the total time expended.

Researchers must be trained for transect surveys. Training, however, no matter how rigorous, may not be an adequate substitute for hands-on experience with the methods and the target species. Depending upon the target species and experiences of those involved, surveying one or two (or more) practice transects may be advisable to ensure that all members of a team are equally adept at all phases of transect sampling. Prior knowledge of the distribution and habits of target species by all members of the research team is essential. In many cases, all personnel involved in transect surveys for difficult-to-detect species and/or endangered species must meet specific professional requirements. Additional training/experience beyond the acceptable minimum may be necessary if observers are to achieve a level of confidence necessary to meet the specific research objectives.

Multiple-visit surveys rather than single-visit surveys are generally the norm with distance or standard transect sampling. Multiple visits allow for repeated and testable sampling, which can then be used to refine and/or calibrate survey methods for the highest level of accuracy. In contrast, single-visit surveys are not likely to generate data of the greatest utility and/or rigor, and we recommend that transect surveys not be used unless multiple surveys at a site are anticipated.

Data Treatment and Interpretation

Transect surveys almost always underestimate population size and density. The reality is that during any given survey a proportion of the target species will not be detected. For this reason mark-recapture or mark-resighting studies, which are more costly, labor intensive, and time consuming than traditional transects, provide more precise estimates of population size or density. The two approaches may be combined, however. For example, by marking a subset of animals and then conducting transect surveys, one can estimate the proportion of animals detected during a given survey. It is essential that the marks not be visible to the observer, however. If they are, it is likely that observers will cue in on the marks and, therefore, have different detection probabilities when searching for marked or unmarked animals. This is an acute problem with color marking (anoles for example). Once the proportion of animals is determined, one can then adjust data derived from transects to obtain more realistic estimates of population size or density.

In summary, transect surveys are a useful way of standardizing effort and location for long-term studies that include determinations of presence/absence, relative density, and abundance of one or more target species. Transects have been used for decades, and they have been refined significantly over that time. As long as certain assumptions are met, powerful statistical tests allow investigators to interpret their transect data with great confidence. In general, transects can be extremely efficient and cost-effective, and if the proper assumptions are met, they are a powerful method for obtaining a great deal of information about a target species.

Pitfall-Trap Surveys

Robert N. Fisher and Carlton J. Rochester

Many reptile species can be rather inconspicuous, making them difficult to survey. Consequently, the ecology and habitat affinities of such species are not well known. Yet before researchers and land managers can answer ecological questions and address the management needs of a local herpetofauna, they must know which reptiles and amphibians occur in the area of interest. If no reliable faunal list is available, then the researchers and managers need to carry out a field survey that detects all or most of the species in the area with minimal sampling bias. One technique that can meet these criteria employs drift fences with pitfall and/or funnel traps. Drift fences are barriers that intercept and guide small terrestrial animals into pitfall or funnel traps placed along their lengths. Pitfall traps are open containers buried so that their upper edge is level with the ground. As small terrestrial animals move across the ground, they fall into the containers and are unable to get out. Funnel traps are elongated traps that have funnels at one or both ends. Animals pass into the trap easily, entering initially through the large end of the funnel, which faces outward. Once inside the traps, however, the animals have difficulty finding their way out through the small end of the funnel and are trapped.

We initiated a large-scale pitfall-trapping study in coastal southern California in 1995 using these traps, which have proven effective at sampling a high diversity of reptiles and amphibians, invertebrates, and small mammals (Fisher and Case 2000; Case and Fisher 2001; Laakkonen et al. 2001; Fisher et al. 2008). In this section we describe the elements of our pitfall-trapping protocol in detail, with a brief account of how to process reptiles (lizards, crocodilians, snakes, and turtles). We also deal with trap-array design and installation, materials used, personnel, equipment, and logistics requirements, sampling schedules, operating procedures, and other pertinent topics.

Background and Justification

Reptiles can be difficult to locate because of their small sizes, secretive behaviors, and cryptic coloring. In such instances, researchers employ numerous techniques, singly or in combination, and with or without drift fencing. The methods include any of the ones described in this chapter, but especially road running, time-constrained searches, quadrat searches, and surveys of woody debris and cover boards, as well as pitfall trapping and funnel trapping (Scott 1982a; Heyer et al.1994a). Comparison studies of different sampling techniques have revealed that each technique has its own advantages, disadvantages, and set of sampling biases (Campbell and Christman 1982; Vogt and Hine 1982; Corn and Bury 1990; Rice et al. 1994; Fair and Henke 1997a; Jorgensen et al. 1998; Ryan et al. 2002). In order to compare data collected over multiple sites or times directly, it is important to use a standardized sampling method that minimizes the amount of observer bias while maximizing the number of species documented. Time-constrained searches, quadrat searches, and road running can introduce significant amounts of bias if the observers have different skill levels. We believe that the most effective technique for trapping a wide variety of species in various habitats with the least amount of observer bias is the pitfall trap/funnel trap/drift fence combination (Case and Fisher 2001; Ryan et al. 2002). To validate the effectiveness of this technique, Case and Fisher (2001) compared results from their use with those from several other survey techniques. At the same sites and times that pitfall traps were operated, professional herpetologists conducted timed walking transects, timed visual surveys of search plots, high-intensity herpetological searching, and passive-observing surveys. None of these techniques determined diversity or relative abundance of the local herpetofauna as completely as did the pitfall traps (Case and Fisher 2001). Gibbons and Semlitsch (1982) have discussed advantages and limitations of the drift fence technique.

Pitfall traps, funnel traps, and drift fencing have been used to trap reptiles since the 1940s. In early studies traps were typically placed singly in quadrats or along a simple linear fence (Imler 1945; Fitch 1951; Banta 1957, 1962; Medica et al. 1971). The shape of the trapping array has since been adapted for specific habitat types and specific study objectives. Traditional fences with large pitfall traps are effective for sampling squamate reptiles (Imler 1945; Gloyd 1946; Woodbury 1951, 1953; Nelson and Gibbons 1972; Semlitsch et al. 1981; Enge 2001; Ryan et al. 2002). Fences made of various materials with or without pitfall traps are effective for sampling turtles (Bennett et al. 1970; Gibbons 1970; Wygoda 1979; Burke et al. 1998). Funnel traps placed alongside drift fences are effective at capturing both amphibians and reptiles (Enge 2001). In order to study population and community dynamics around ponds and ephemeral aquatic habitats, fenced arrays have been modified to encircle the water, thus catching all incoming and outgoing species (Storm and Pimental 1954; Gibbons and Bennett 1974; Dodd 1992a; Dodd and Scott 1994). Terrestrial arrays with trap arms extending from a center pitfall trap (an "X" or "Y" shape) are more effective than linear arrays (Campbell and Christman 1982).

The drift fence–pitfall method has also been employed to study habit use (Bostic 1965; Loredo et al. 1996) and population dynamics of individual species (Pearson 1955; Parker 1972) and communities (Storm and Pimental 1954; Gibbons and Bennett 1974; Dodd 1992a). More recently, it has been used to address current ecological issues. For example, arrays have been replicated over multiple sites to look at variations in species occurrences within and among habitats (DeGraaf and Rudis 1990) and to study the effects of human-induced impacts on the relative abundance and diversity of herpetofaunas (e.g., Jones 1981; Rudolph and Dickson 1990; Burkett and Thompson 1994; Ireland et al. 1994; McCoy and Mushinsky 1994). This type of presence/absence data is excellent for use in species-distribution modeling for appropriate taxa (see Franklin et al. 2009).

Many studies have included only a single type of trap, been of limited duration, and/or were conducted over a localized geographical region. We used a standardized array of pitfall traps, funnel traps, and drift fencing to perform long-term

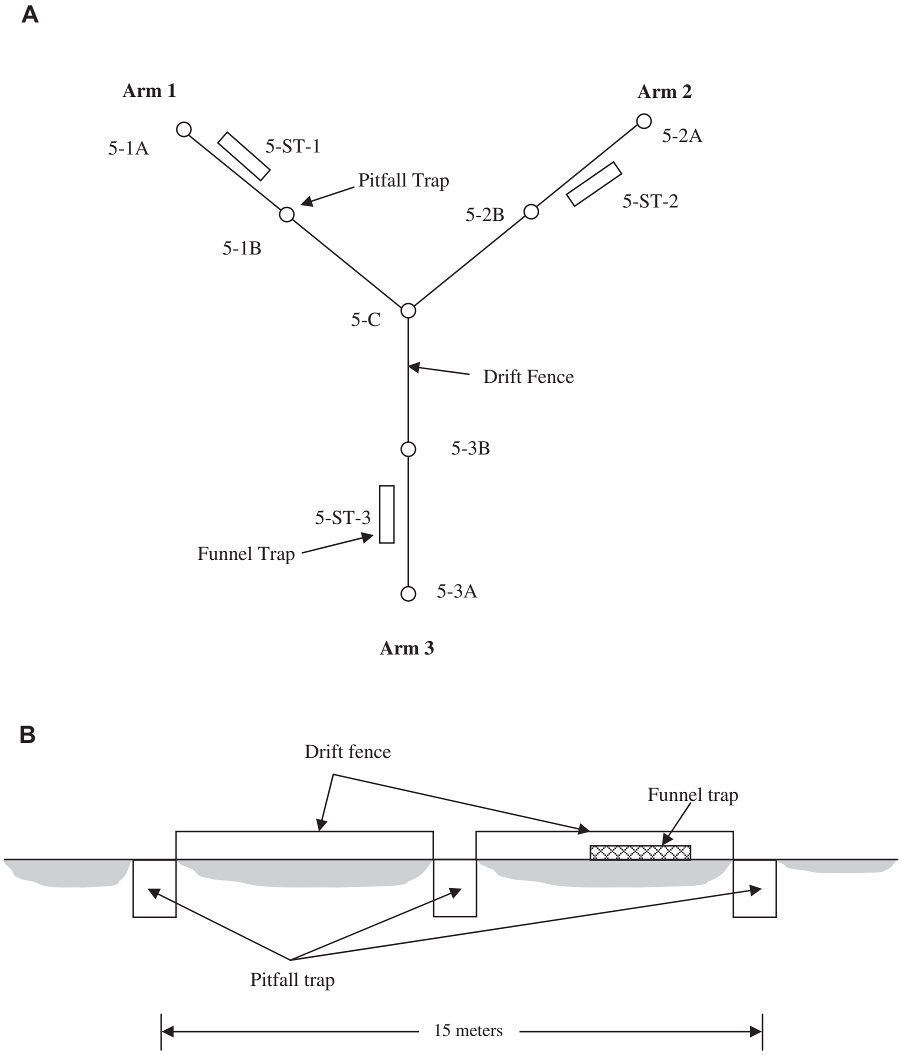


FIGURE 75 Pitfall-array design. (A) Overhead view of array design, showing pitfall traps, funnel traps (ST), and drift fences. In this example, the traps are numbered as if at array 5 of the study site. (B) Side view of a single arm, indicating the relative positions of the three trapping elements.

research over a wide geographic area in southern California, with replicates over site localities, habitats, and environments (Fisher and Case 2000; Fisher et al. 2002; Fisher et al. 2008). The large scope of the trapping effort enabled us to evaluate the effects of both local landscape features and variables such as latitude, altitude, and climate characteristic of larger geographic areas on herpetofaunal assemblages (Fisher and Case 2000). We have used the data to study the autecology of sensitive species, effects of habitat fragmentation and introduced species on native wildlife, regional patterns of herpetofaunal diversity, and historic versus current species distributions, as well as for species-distribution modeling (Case and Fisher 2001; Laakkonen et al. 2001; Fisher et al. 2002; Franklin et al. 2009).

Array Design

Array design refers to the positioning of the traps and drift fences relative to each other within a study area. Three-arm arrays yield results comparable to those with four arms (Corn 1994) but require less material and time to construct. Our array design consists of three 15-m drift fences radiating from a central pitfall trap (Fig. 75) at approximately 120-degree intervals. Additional pitfall traps are placed at the middle and at the distal end of each arm of fencing. One funnel trap is consistently placed on the right side of each arm (to ensure that sampling procedures are comparable among arrays) approximately halfway between the middle and end pitfall traps when looking from the center trap toward an end trap. These traps work best for large snakes and are often referred to as "snake traps" (ST).

Each array at a study site is assigned a number, and then each arm of the array is assigned a number (1, 2, or 3) in a clockwise direction, with 1 being arbitrarily designated (usually the arm first encountered when approaching the array). The pitfall containers are labeled A, B, and C for outer, middle, and center buckets, respectively. Each trap, thus, has a unique identifier consisting of the array number followed by the arm number and then its position letter. For example, the middle container of arm 3, at array 5, would be pitfall trap 5-3B. The center container of array 5 would be identified simply as 5-C. The funnel traps are identified by the array number and the number of the arm along which they lie. For example, the funnel trap located along arm 2 of array 5 would be identified as 5-ST-2 (Fig. 75).

Ancillary Data

WEATHER

Because we were concerned about temperature attributes with which reptile activity might be correlated, we recorded ambient temperature at 1 m above the ground at each study site during the sampling period. Temperatures were taken daily with a maximum-minimum thermometer or every 15 min with an automated datalogger. We also recorded the weather conditions each day of each sampling period as sunny, foggy, overcast, or with precipitation. Investigators should place the weather station at the location that best represents the study site. Some study sites may require more than one weather station, depending on the topographic diversity in the area and the number of different microclimate zones it encompasses. Portable weather stations can be installed at a study site when arrays are opened and then removed at the end of the sample period and moved to the next site. Investigators interested in more comprehensive information about weather should consult "Climate Data and Seasonality" and "Automated Data Acquisition," in Chapter 4.

VEGETATION

Some studies require data on vegetation and/or habitat characteristics, for example, as a means of characterizing microhabitat use. We sampled vegetation only once at each array unless it changed substantially (e.g., due to disturbance such as fire) or was directly relevant to the study questions, in which case we repeated the survey or surveyed more regularly. There are multiple ways to survey vegetation; investigators should review methods used in other studies with similar goals and from similar habitats. We surveyed vegetation at each pitfall trap array using a point-intercept transect technique (Sawyer and Keeler-Wolf 1995) and recorded information onto standardized paper or electronic data forms. We also recorded slope, aspect, GPS coordinates, and date at each array site.

Array Materials and Trap Construction

Below we describe the materials needed to construct and operate a pitfall array and provide instructions for making individual traps. We use metric units for measurements except for materials that are commonly sold in standard English units.

DRIFT FENCING

A variety of materials, including clear roll plastic, silt fencing, wooden boards with bronze window screen, aluminum flashing, hardware cloth, and galvanized metal can be used to make effective drift fencing; investigators should choose the material that best suits the substrate(s) and weather conditions in the study area (Milstead 1953; Storm and Pimental 1954; Pearson 1955; Gibbons and Semlitsch 1982; Campbell and Christman 1982; Bury and Corn 1987; Murphy 1993; Enge 1997b; Jorgensen et al. 1998; Zug et al. 2001). For example, a porous material (such as hardware cloth) is best for areas with high winds, which can tear out solid fencing. Gibbons and Semlitsch (1981) considered aluminum flashing superior to other fencing materials because its smooth surface makes it difficult for animals to bypass and because it neither deteriorates nor rusts. However, aluminum flashing is unsuitable for the extreme topography in which we commonly work; instead, we use 30-cm-tall nylon shade cloth, even though some animals can climb over and cross it. Each array arm is 15 m long (7.5 m between each pair of pitfall traps). The drift fences are secured to from 10 to 15 upright wooden stakes (1×2×24 in, or 2.4×4.8×61 cm) per 15-m arm of fencing, with heavy-duty staples (see "Array Installation," below).

PITFALL TRAPS

Various containers, such as 5-gal (= 19-liter) plastic buckets, coffee cans, metal buckets, lard cans, pottery water containers, and 55-gal (= 208-L) drums, can be used as pitfall traps (Pearson 1955; Banta 1957, 1962; Gibbons and Bennett 1974; Gibbons and Semlitsch 1981; Campbell and Christman 1982; Vogt and Hine 1982; Yunger et al. 1992; Corn 1994; Zug et al. 2001). Larger containers generally capture more animals than small ones (Vogt and Hine 1982), because the likelihood of an animal escaping decreases with increased container depth. Smaller containers may be useful, however, if the target animal(s) is small (Shoop 1965; Gill 1978; Douglas 1979). Additionally, fitting a plastic, funnel-like collar inside the top of a pitfall trap (with the small-hole end inserted into the trap) will prevent the escape of some animals.

Containers should be buried with the rim flush with the ground. Small drain holes punched in the bottom will minimize flooding if it rains while the traps are open. We typically use white plastic 5-gal (19-L) buckets. At desert sites, however, we use 6-gal (23-L) buckets because the increased depth provides increased insulation from heat. Crawford and Kurta (2000) found that black plastic buckets trapped frogs and shrews more effectively than white plastic buckets. However, internal temperatures may be higher in black buckets exposed to intense sunlight for long periods, which could increase animal mortality and damage the containers. Dodd (1992a) used slanted pegboards to partially shade black buckets in Florida.

PITFALL-TRAP COVER

All pitfall traps should have some form of cover when open to shield animals from the elements. We set an inverted lid on top of the trap with wooden spacers (Fig. 76). This design prevents most litter, sunlight, and precipitation from entering the open bucket, while allowing sufficient space for small animals to enter.

To construct a top, we cut three 6.4-cm-long pieces of 2-×2-in construction-grade wood at a 35° angle. We attach these pieces, facing outward, around the top of the bucket lid using 1.25-in drywall screws with SAE (Standard American Engineers) #10 washers. We then make vertical cuts at 15-cm intervals around the outer perimeter of the bucket lid, which makes opening and closing the traps easier. When the traps are open, the lid is turned over with the spacers resting on top of the bucket (Fig. 76). Animals and wind occasionally remove lids

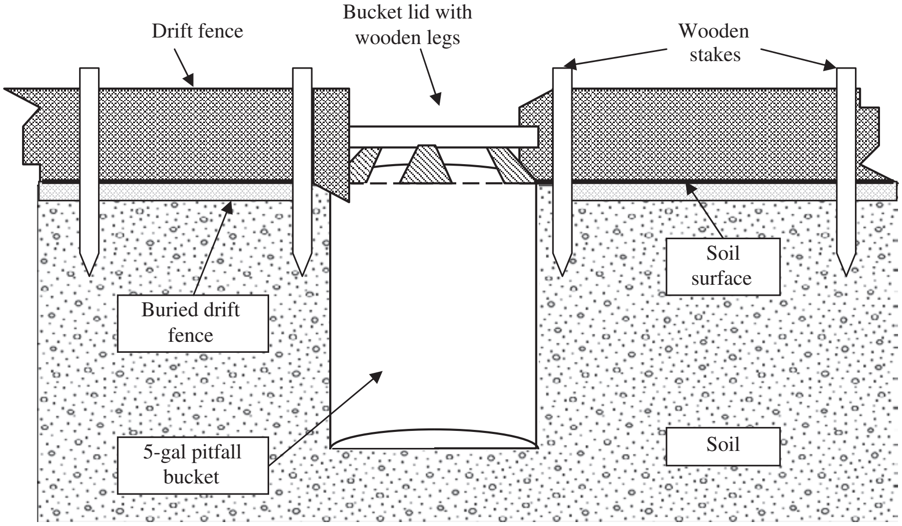


FIGURE 76 Underground cut-away view of a pitfall container without accessories. Each pitfall trap consists of a 5-gal bucket buried so that its top rim is even with the surface of the ground. Three wooden legs are attached to the outer side of the bucket lid. An inverted lid standing on its legs acts as a raised cover (roof) over the pitfall bucket. A flap of the drift fence continues past the last fence post on either side of the bucket so that as little gap as possible occurs between the fence and the bucket.

from open containers. Bungee cords can be used to keep the lids attached. We next drill three holes with diameters similar to that of the bungee cords into the sides of the bucket. The holes should be evenly spaced and approximately 8 cm below the rim. Next, three 12-in bungee cords are cut in half. The cord ends are fed through the holes from the inside of the container and a knot is tied at the end of the cord on the outside. The hook ends of the cords are pulled from the container and fastened to the lid while it rests on the wooden feet. The tension on the cords should be such that removing the lid from its resting position requires considerable force. Wooden boards that are attached to the pitfall containers with bolts and eye-sockets can also be used to keep container lids on while the pit container is being sampled (Fellers and Pratt 2002).

COVER WITHIN PITFALL TRAPS

Cover for captured animals should be provided within the pitfall traps. We use a 6 in long piece of 1.5-in-diameter PVC pipe and an 8 in long piece of 1-in-diameter PVC pipe. We place some form of insulation, such as synthetic batting or foam material, in the PVC pipes if small mammals are likely to be captured. We place a section of closed-foam pipe insulation within the 6-in piece of pipe. We also recommend placing a wet sponge in the pitfall trap to help keep amphibians hydrated. The sponges should be wetted daily when traps are opened. We discontinue the use of sponges in southern California during the dry months, because they usually attract ants, and large number of ants will usually kill or seriously injure most small vertebrates in the traps.

FUNNEL TRAPS

A variety of materials can be used to make funnel traps but 0.125-, 0.25-, and 0.33-in mesh hardware cloth have been the materials of choice in herpetological studies (Imler 1945; Gloyd 1946; Fitch 1951; Milstead 1953). The traps should be sturdy yet lightweight. We used 0.25-in mesh hardware cloth.

To construct funnel traps, one first cuts a 100-ft roll of 36-in-wide hardware cloth into 18-in-long sections. For each cylinder body, one 36-×18-in section of hardware cloth is rolled into a 36-in-tall by ~5.75-in-diameter cylinder held together with hog rings or plastic zip ties (Fig. 77). Metal hog rings persist longer than plastic zip ties, which are good for only 1 year under field conditions in southern California. To make funnel ends, a 24-in-diameter circle of the same hardware cloth is cut into four equal sections (Fig. 78). Each piece is rolled into a funnel and serves as one end of the trap. The small opening of the funnel should be approximately 2 inches in diameter, which will allow animals, including large snakes, to enter the cylinder. The small ends of the funnels fit into the ends of the cylinder, with the large ends pointing out. We cut the overhanging material to the edge of the cylinder body in five places to form flaps, which are then folded over the edge of the cylinder so that the funnels fit onto it snugly (Fig. 79). Finally, each funnel is fastened to an end of the cylinder with two medium-size binder clips (Fig. 80). Trimming all sharp points and edges during the cutting process helps to prevent injury to captured animals and field personnel. In the field, the traps are placed with the seams of both the body and end cones facing upward to prevent injury to the animals from rough edges of the hardware cloth.

Funnel traps should be covered at all times to prevent excessive exposure to sunlight and precipitation. We use shingle boards as covers (Fig. 81) and place a board beneath the trap to shield it from extreme substrate temperatures. In the desert and in high wind areas, 2-×2-ft squares of ¾-in plywood substitute for shingle boards. We place a 6-in-long piece of 1.5-in-diameter PVC pipe containing some form of insulation (e.g., synthetic batting or foam material) into the trap for additional cover.

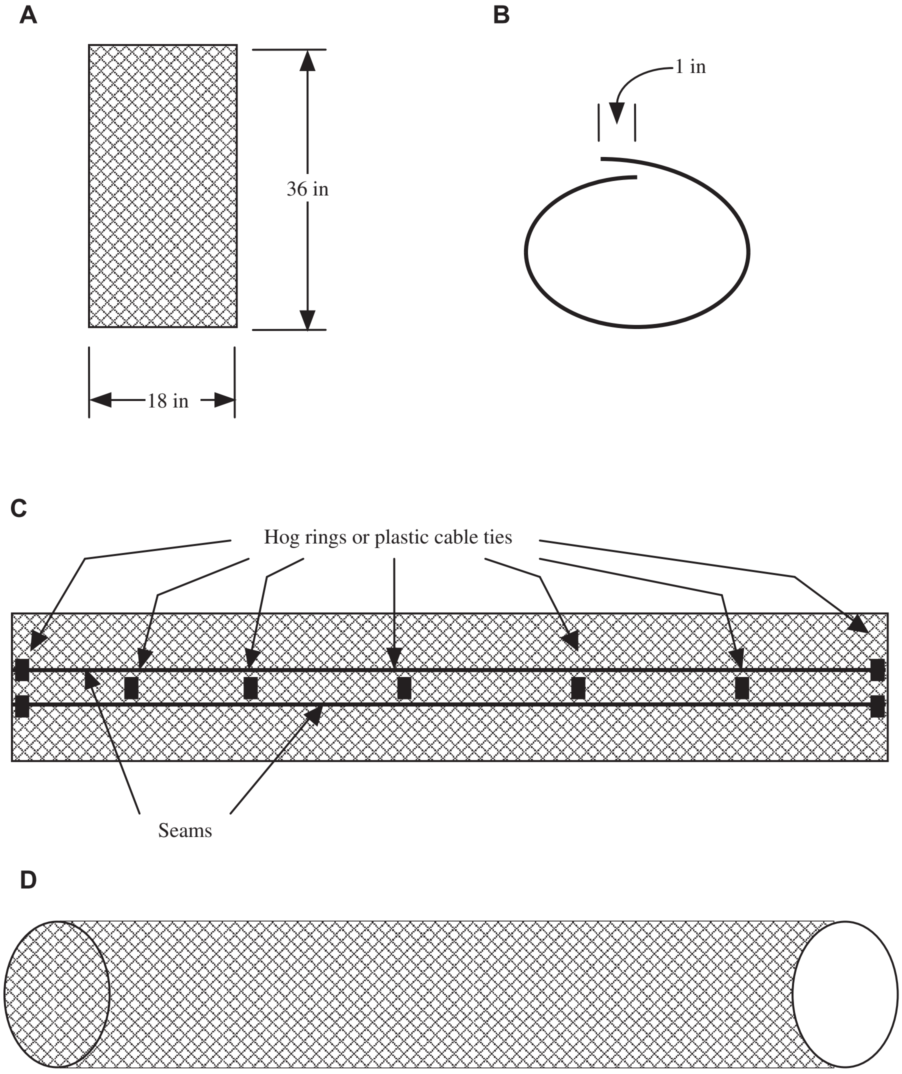


FIGURE 77 Funnel-trap assembly (step 1). (A) 0.25-in mesh hardware cloth is cut into 18- × 36-in sections. (B) Each piece is rolled into a cylinder, with 1-in overlap. (C) The cylinder is secured using hog rings or plastic zip ties. (D) The result is a 36-in-long cylinder that forms the body of the trap.

FUNNEL TRAPS WITH PITFALL-TRAP RETREATS

In areas with extreme temperatures, such as deserts, it may be necessary to associate a substantial secondary retreat with each funnel trap. This can be done by installing a 6-gal pitfall trap (with lid) in the spot where the funnel trap will be located (Fig. 82). Prior to rolling the hardware cloth into a cylinder for the funnel trap, a hole of appropriate diameter is cut into the center of the cloth with a sharpened steel pipe or other tool. The trunk of a 1.5-in-diameter PVC-pipe T-joint is placed through the hole in the hardware cloth, and the funnel trap is completed with the top of the T-joint inside the cylinder. An 8-×1-in-diameter piece of PVC pipe is inserted into the trunk of the T-joint (Fig. 83). The funnel trap is then attached to the buried bucket with the pipe extending down from the bottom and passing through a hole cut in the lid of the buried bucket. The holes in the funnel-trap cylinder and the bucket lid should be just large enough to accommodate the pipe, which is secured to the funnel trap with wire. Captured animals seek cover in the buried bucket via the T-joint pipe.

Array Installation

PERSONNEL, EQUIPMENT, AND LOGISTICS

The time required to install a set of arrays varies with the substrate and terrain. In general, a four-person team can install a single array in about1 hour, not including the time required to load/unload equipment and drive to the site. Usually no more than five arrays can be installed by a four-person team in a single workday regardless of the study site. The equipment needed for a four-person team to install pitfall-trap arrays is listed in the Table 21.

Before installing an array, an investigator must obtain permission from the owner of private land or the administrator of public land, as well as state, federal, or other (e.g., archeological

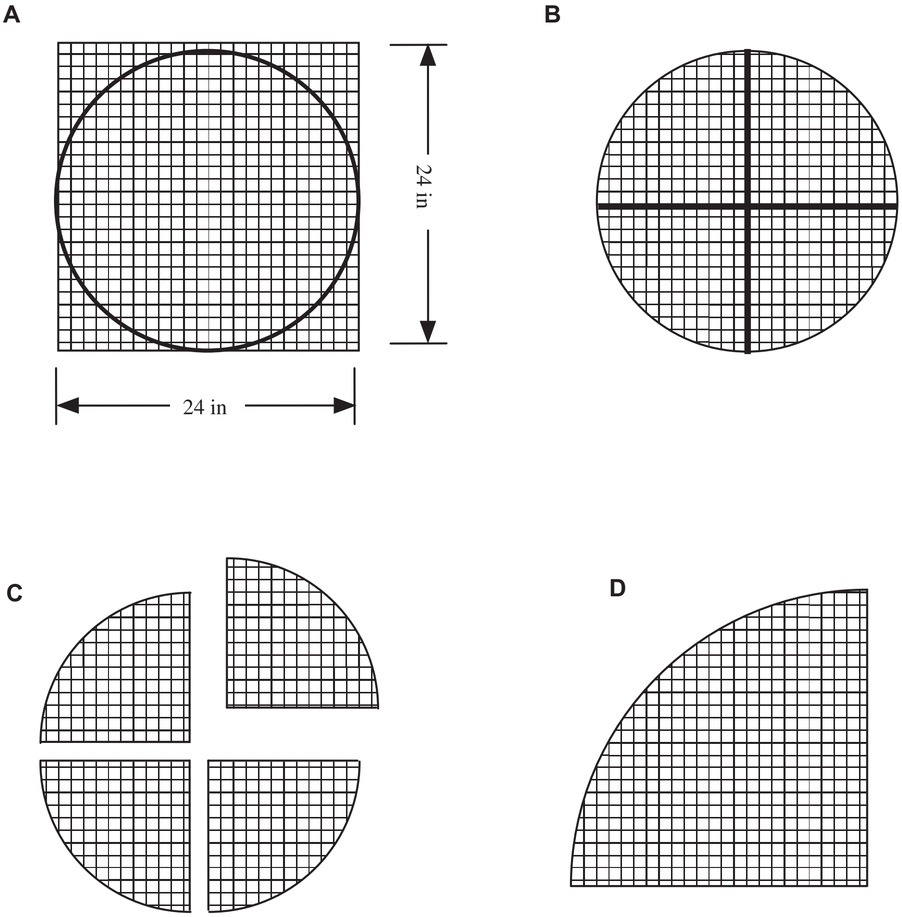


FIGURE 78 Funnel-trap assembly (step 2). (A) 0.25-in mesh hardware cloth is cut into 24- × 24-in pieces. (B) Each piece is trimmed to form a circle with a 24-in diameter. (C) The circle is cut into four equal sections. (D) The result is a quarter circle with a 12-in radius.

clearance or endangered species) permits to carry out the study on the target species. Generally, investigators should select more sites than necessary in case the use of one or more is restricted or prohibited.

INSTALLATION OF PITFALL ARRAYS

During site reconnaissance, investigators should flag the spot the center bucket will occupy in each pitfall-array location. Someone then measures the first 15-m array arm from that point, marking the 7.5- and 15.0-m points on the ground with bucket lids. The remaining two arms are also measured from the center trap at approximately 120-degree intervals. The fence arms should be as straight as possible but can bend around large rocks, trees, and other barriers.

At the pitfall-trap locations, crew members dig holes using tools appropriate for the substrate. The holes should be just large enough to accommodate the pitfall containers, unless the substrate does not drain easily. In that case, the holes should be deeper than the buckets and the extra depth filled with rocks. The space should accommodate the water drained from the containers should they become flooded. If holes are not drilled in the bottom of a bucket, then it will pop out of the ground if it is closed during a rain event. If a bucket is open but without holes, it will flood, killing most of the trapped animals.

Before the buckets are buried, the rims of the bucket lids should be clipped vertically in four or five places to make removing them easier when opening the traps. The buckets are placed in the holes with their covers on, and dirt from the excavation is packed in the space around them until the top of the bucket is flush with the ground. The soil must be compacted firmly so that stakes beside buckets have a solid foundation. Next, 3- to 5-in-deep trenches are dug where drift fencing will be installed along the length of each arm. To install the drift fence, one first lays the appropriate length of fencing along the trench. Typically, 10 to 15 stakes are needed per array arm. For each array arm, one stake each is placed as close to the center and end containers as possible (to maximize trapping efficiency), one stake is placed on either side of the container in the middle of the array arm, and stakes are placed at ca.1-m intervals along the fence between the containers. If an array arm changes direction or makes a turn, a stake is placed at the inside of each turn. If an array is built on a slope, the stakes are positioned on the downhill side of the trench for stability. The stakes are placed along the edge of the trench and pounded into the ground deep enough to be secure but remain above the top of the drift fence.

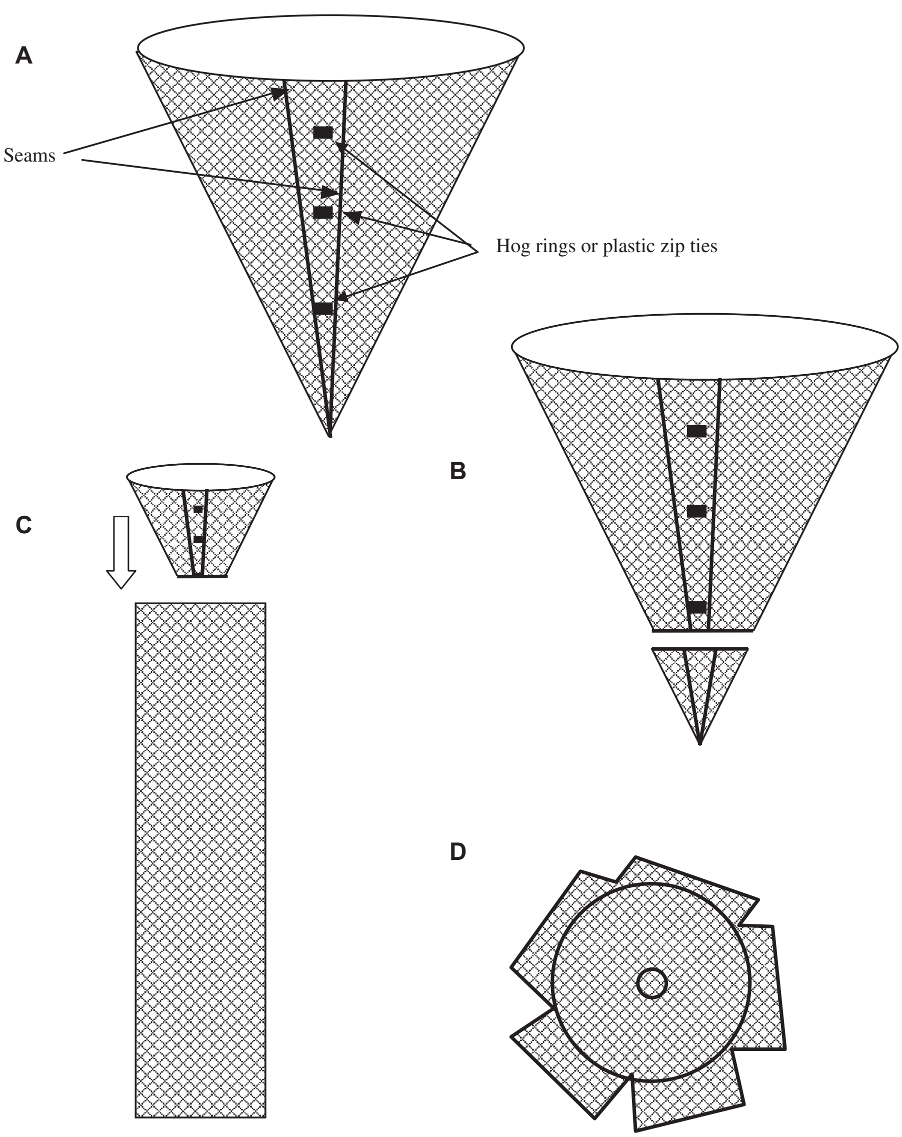


FIGURE 79 Funnel-trap assembly (step 3). (A) The quarter circle of hardware cloth is rolled into a cone and secured using hog rings or plastic zip ties. (B) The point of the cone is cut off to create a 2-in opening. (C) The cone is placed onto the body of the funnel trap, small end first. (D) The edge of the cone is clipped to create tabs so that it fits snuggly on the end of the body of the funnel trap.

Once the stakes are installed, the drift fencing is placed in the middle of the trench against the stakes and attached to them. The fence should be anchored to the end stakes adjacent to the buckets first. A 4- to 6-in horizontal cut is made into the end of the fencing ca. 2 in up from the bottom. The resulting strip is wrapped around the stake and stapled in place so that the top (unburied) portion of the fence extends straight to the edge of the bucket and beyond, forming a flap that helps to guide animals into the pitfall traps. Starting with the stake at the container in the center of the array, and keeping the fence as taut as possible, the installer fastens the drift fence with heavy-duty staples to each stake working from bottom to top. At the container in the middle of the array arm, the fence is cut such that the fence edge extends just above the edge of the container and is attached as described for the first end stake (Fig. 76). The fence begins again on the distal side of the middle container and is fastened to all the remaining stakes. The procedure is repeated to install fences along the other two array arms. The excavated substrate is replaced and packed along the drift fencing such that the bottom of the fence is buried completely and the rims of all of the pitfall containers are flush with the ground. Funnel traps are placed along the right side (when viewed from the center bucket) of each arm between the middle and distal pitfall containers (Fig. 75). All buckets remain closed and funnel-trap ends removed until sampling begins.

Array Operation

SURVEY SCHEDULING

The timing of surveys depends on the research objectives of the study. For instance, opening traps after a rain will maximize captures of some species but decrease captures of others. However, opportunistic trapping (i.e., triggered by an irregularly

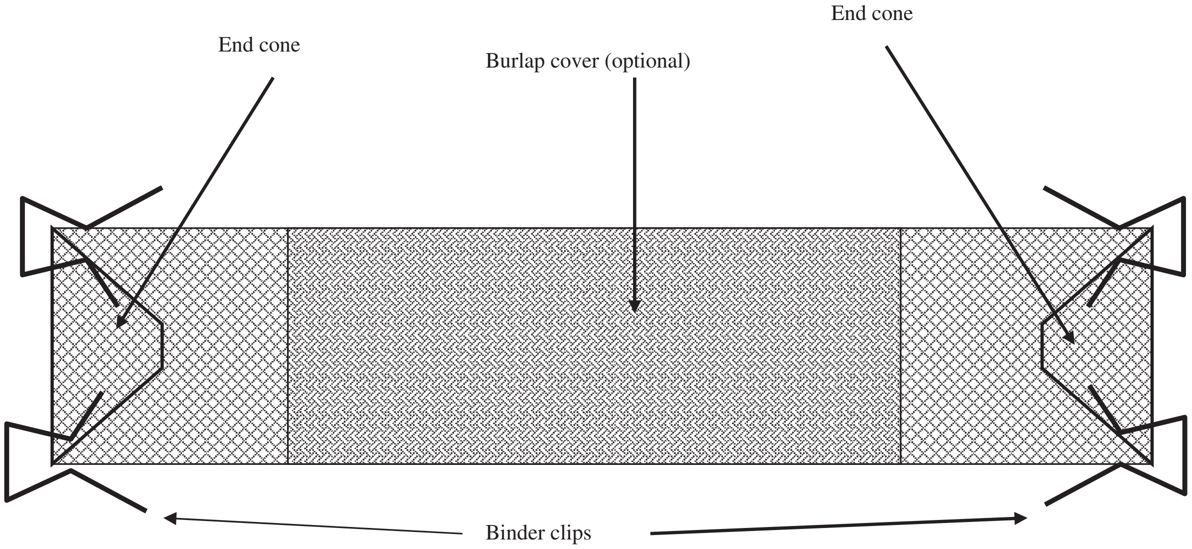


FIGURE 80 Funnel-trap assembly (step 4). A completed funnel trap, or snake trap, consists of one cylindrical body segment and two end cones that are held in place with four medium-size binder clips. For added cover, a piece of burlap cloth may be secured to the outside of the body segment.

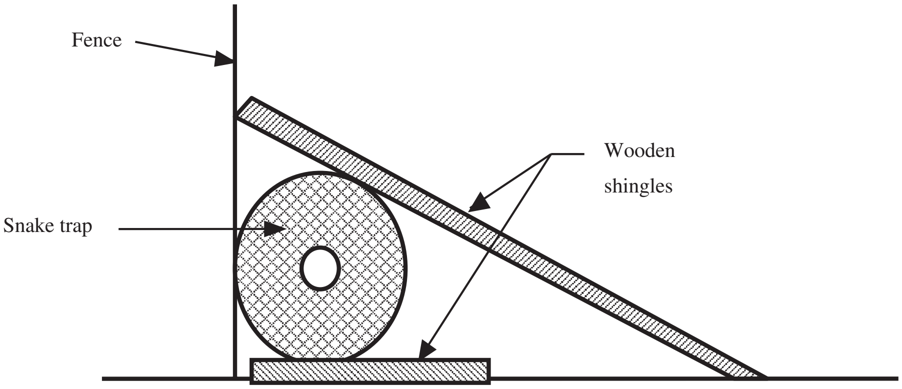


FIGURE 81 Funnel-trap cover. Each snake trap sits on top of a wooden roofing shingle next to the right side of the fence, as viewed from the center bucket, between the "A" and "B" pitfall-trap buckets. Additional wooden shingles are arranged over the top of the funnel trap to provide shade.

occurring event), may be logistically difficult. Rain events can be unpredictable and can interfere with access to the arrays if travel on dirt roads is required. Conversely, continuous trapping reveals seasonal and weather-related variations in animal activity but requires more personnel to check traps and may affect the behavior of resident animals if they are captured repeatedly.

In our protocol, we sample a given study site for 4 consecutive days and then close the traps (i.e., open on Monday, sample daily Tuesday through Friday, close on Friday). This constitutes one sample period. Sample periods for a given site are scheduled every 4 to 5 weeks, resulting in 10 to 12 sample periods per year. This schedule allows for the collection of target information, including seasonal activity patterns of reptiles and other trapped animals. Based on our analysis of species accumulation curves, we recommend that a site be sampled over 3 to 5 consecutive years to increase the probability of detecting rare species (Fig. 84). The schedule also depends upon the number of trapping arrays at a site. We usually place arrays in multiple representative habitats within a site and include replicates within habitat types as funding permits.

Personnel, Equipment, Logistics

In general, a single person can operate 20 to 30 pitfall-trap arrays per day. A single person following the 4-day sampling schedule and working a 40-hour week can operate four sets of 20 pitfall-trap arrays on a rotational basis each month. Some circumstances, such as long distances between arrays or personnel safety concerns, warrant a two-person team.

Each person or team operating pitfall-trap arrays will need a portable field kit (e.g., backpack and/or tackle, tool, or ammunition box) that contains items for processing animals and recording data (see Table 21).

SAFETY PRECAUTIONS

If venomous snakes occur in trapping areas, persons operating the traps should wear some form of leg and ankle protectors, such as "snake chaps." Venomous snakes in pitfall traps can be removed carefully with a snake stick, if they are unable to exit

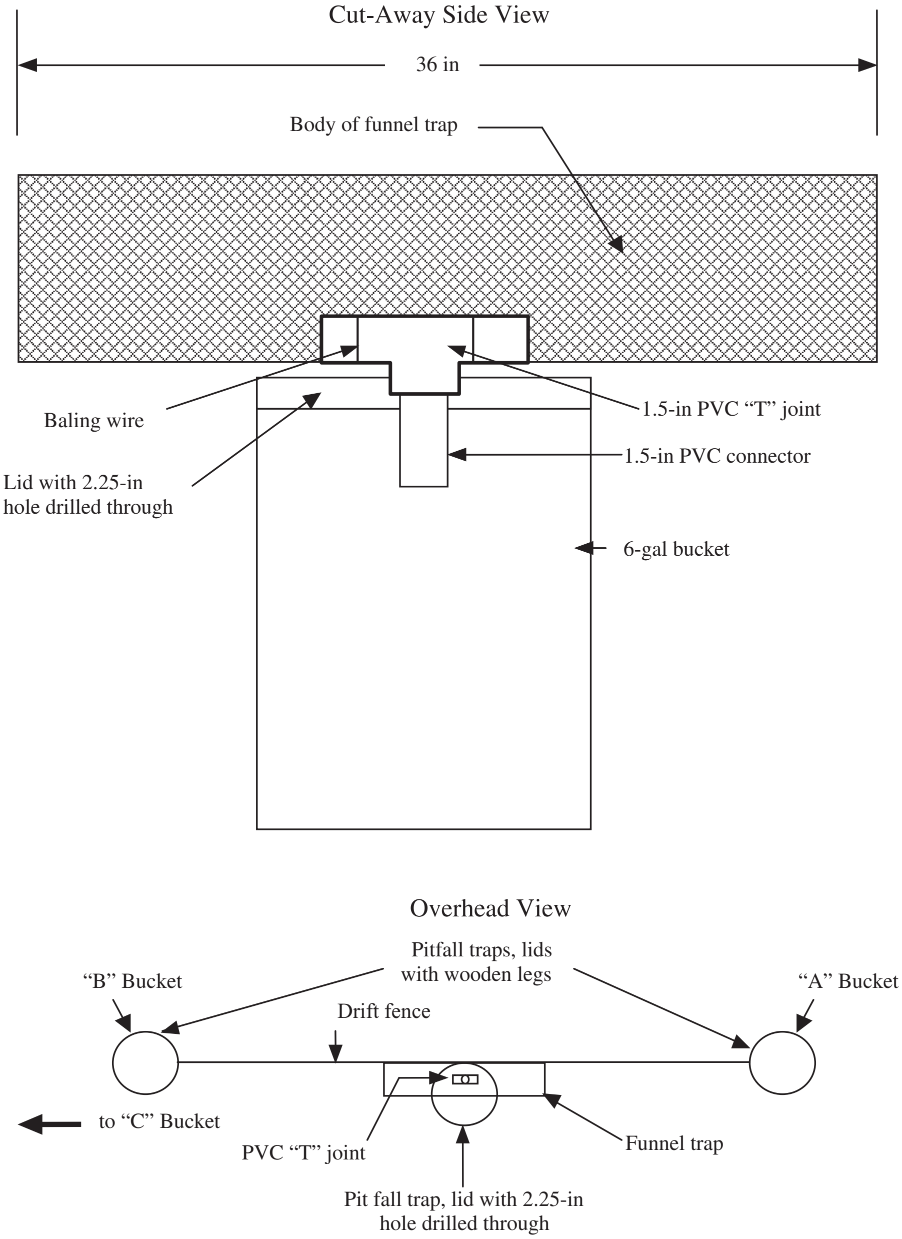


FIGURE 82 Desert funnel trap. In desert environments, a funnel trap can be modified to incorporate a pitfall trap that provides a cooler retreat for a trapped animal. An animal in the funnel trap enters the pitfall trap by means of a PVC "T" joint.

on their own. Venomous snakes in funnel traps can be removed by carefully removing one end, tipping the snake out a few meters away from the array, and allowing it to move off away from the researcher. The handling of venomous snakes is described in Chapter 8, "Dealing with Live Reptiles." Other stinging or biting vertebrates or invertebrates also fall in traps and warrant careful handling.

Fieldworkers should take precautions in areas where communicable diseases, such as hantavirus or bubonic plague, are likely or known to occur in rodent populations. Rodents often enter both pitfall and funnel traps, so persons operating traps should wear latex and/or thick leather gloves and some form of respiratory protection. Project leaders should check with local public health authorities before initiating fieldwork. Traps should be washed in soapy water periodically; they can be sterilized with a dilute (2–5 %) solution of sodium hypochlorite (household bleach = 5.25%) solution but then must be rinsed thoroughly.

Employing Traps

A sample period starts when the pitfall and funnel traps at each array are opened. Dirt and debris are removed from the container with a spatula or cup. The lids from the pitfall containers are inverted so that their wooden "feet" sit on the container rim. Precleaned (washed, sterilized, thoroughly rinsed) pipes and sponges are placed in each trap as it is opened. A shingle

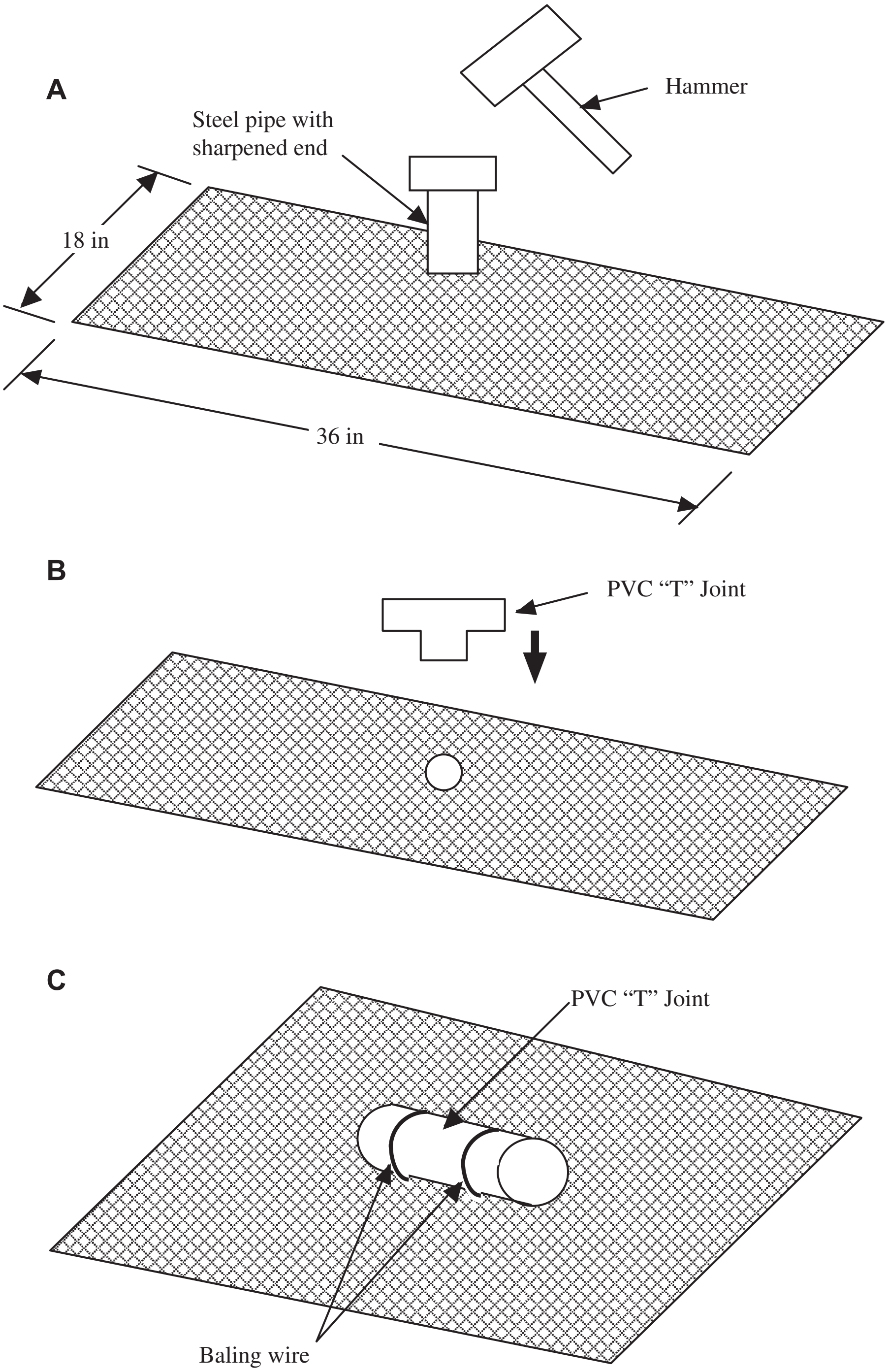


FIGURE 83 Desert funnel-trap assembly. A hole (A) is punched into the center of an 18- × 36-in section of 0.25-in-mesh hardware cloth to accommodate the 1.5-in-diameter PVC "T" joint (B), which is secured in place with baling wire (C).

board is placed under each funnel trap, a PVC pipe with insulation is placed inside, and the entire trap is covered with shingle boards. A funnel trap is "opened" by fastening a funnel entry to each end of the cylinder body using the binder clips.

Traps are checked on the 4 consecutive days after opening, usually during the early morning. The lids are removed from the pitfall traps, and the insides of the containers (including under the sponge), the PVC pipes, and any debris are visually inspected. During the wet season, sponges are moistened daily. Funnel traps are first visually inspected for venomous snakes and then picked up by one end so that the insides of the trap and the PVC pipe can be inspected. This usually does not require removing an end funnel. If a funnel trap is connected to a pitfall container (Fig. 82), the trap should be lifted so that the inside of the container can also be inspected. All of the arrays at a study site should be checked and all animals processed and released before daytime temperatures reach levels that could result in animal mortality. If a fast-moving snake

TABLE 21

Materials, Supplies, and Equipment Needed to Build, Maintain, and Sample a Pitfall-Trapping Array

General

- Vehicle with 4-wheel drive (if necessary) to transport the installation team and equipment to and around the study site
- Equipment to remove brush, cut trails, and clear space for array arms

Construction Supplies and Equipment

- Funnel trap
  - 1.25-in (32-mm) binder clips
  - 0.25-in (6.4-mm) mesh hardware cloth, 36-in × 100-ft roll (91.55 cm × 30.5 m)
  - 0.25-in (6.4-mm) mesh hardware cloth, 24-in × 100-ft roll (60.7 cm × 30.5 m)
  - PVC “T” joint, 1.5-in (3.8-cm) SSS for desert sites
  - Hog ringer pliers
  - Steel hog rings
  - Shake shingles, medium untreated or ¾-in (1.9-cm) plywood for desert sites
  - Tin snips to cut hardware cloth and bucket lids
  - Baling wire
- Drift fence
  - Shade cloth, 12-in (30.5-cm) tall
  - Heavy duty staple gun and staples to attach fencing to stakes
  - Scissors (or appropriate tools) to cut drift-fence material
  - Wooden stakes, 1 × 2 × 24 in. (2.54 cm × 5.1 cm × 61 cm)
- Buckets and lids
  - 2- × 2-in lumber (5.1- × 5.1-cm)
  - 5-gal (18.9-L) buckets
  - 6-gal (22.7-L) buckets
  - Plastic bucket lids
  - 1.25-in (3.2-cm) drywall screws
  - SAE washers, #10
  - Bungee cords for desert site
  - Marker to label buckets and lids
- Tools
  - Gloves for handling animals and site construction
  - 50-m tape measure for array arms
  - Spade/shovel, 2 or 3
  - Wooden support stakes
  - Sledge hammer (3–5 lbs) for pounding stakes
  - Electric drill
  - Phillips-head drill bit
  - 0.125-in. (3.2-cm) drill bit
  - Pry bar/digging bar for areas with rocky substrate
  - Hammer drill, if very rocky, with portable generator
  - Pick axes with flat blade ends to dig trenches
  - Auger (optional) with 14-in bit to dig holes for pitfall containers
  - Pitfall containers (5- or 6-gal buckets)
  - Circular saw
  - 0.5-in (1.2-cm) drill bit for desert sites
  - 2.25-in. (5.7-cm) drill bit for desert sites
- Site operation
  - Cellulose sponges
  - 1.5-in- (3.8-cm-) diameter PVC pipe with foam insulation
  - 1-in- (2.54-cm-) diameter PVC pipe

Field-kit Supplies

- Carrying case
- Data book (e.g., small 3-ring binder or handheld computer)
- Snake hook
- Permanent marker for writing on plastic tissue tubes and bags
- Write-in-rain pen or pencil for recording data
- Snake bags

TABLE 21 (*continued*)

Small forceps
Small tweezers
Straight microscissors
Ruler, small, clear plastic, metric
Data forms
Small resealable plastic bags for weighing small animals
Large resealable plastic or “snake” bags (or pillow case) for weighing large animals
50-ml tissue tubes with 70% ethanol
Fiberboard storage box and dividers
Water bottle for sponges
Small spatula or cup for cleaning debris or bailing water from pitfall traps

Large forceps (12 in)
Large tweezers
Small scissors for clipping toes and scales
Metric tape measure for large snakes
Wildlife field identification guides
Metric spring scales (typically 10-, 30-, 60-, 100-, and 1,000-g scales accommodate most small vertebrates)
Extra snake bags or appropriate animal containers
1.5-ml tissue tubes with 95% ethanol
Pen with waterproof, fade-resistant ink
Ice chest with ice packs

Weather Station

Wood bolt with wood thread on one end and machine thread on the other
Wing nut and washer to fit wood bolt

Max-min thermometer or temperature data logger
2-in-diameter PVC pipe

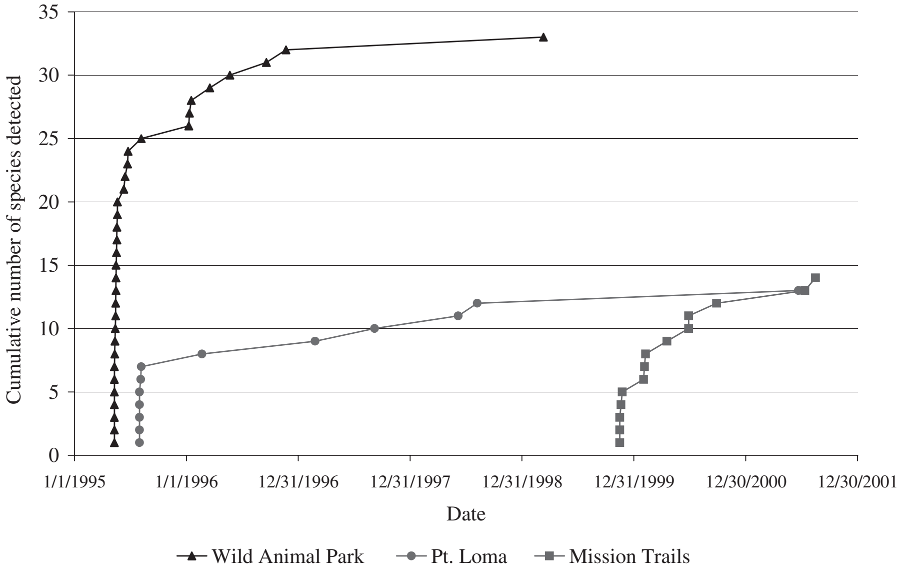


FIGURE 84 Species accumulation curves. The cumulative numbers of species detected by pitfall-array surveys at three study sites in San Diego County, California. The majority of the species present are detected within the first several trapping sessions. Recording less abundant or rare species may require additional trapping effort and/or use of specialized techniques.

(e.g., *Coluber*) or lizard (e.g., *Aspidoscelis*) is captured, the animal should first be emptied directly into a cloth bag or pit trap and then removed for processing. Methods of handling live animals are presented in Chapter 8, “Dealing with Live Reptiles.”

On the last day of a sample period, each trap is closed after its contained sample is processed. To prevent animals from being captured between sample periods, both ends of each funnel trap are removed, and lids to the pitfall containers are tightly attached in the closed position. Placing rocks on the container lids will prevent them from being removed by animals or wind. Large rocks will eventually break through older lids that have become brittle from exposure to the sun. If periods between sampling are long or if arrays are located in sites subject to vandalism, pitfall traps should be checked periodically to ensure that they remain properly closed.

Processing Animals

Processing involves handling, measuring, and marking the animals, and recording relevant data. Although trapped animals are processed and released immediately, incidental mortality occurs occasionally; those animals should be preserved as voucher specimens (see Chapter 7, "Preparing Reptiles as Voucher Specimens"). They may be placed in any airtight container of appropriate size (e.g., 50-ml centrifuge tube). Specimens can be immediately preserved in 10 percent buffered formalin or temporarily stored on ice and later transferred to a freezer. If tissues are needed for genetic analyses, they should be removed before a specimen is preserve in formalin. The tissues should be placed in 95 percent ethanol or other appropriate preservative ("Collecting and Preserving Tissues for Biochemical Analyses," in Chapter 7). Living specimens of representative species should be photographed to serve as vouchers for reports and publications.

FIELD IDENTIFICATION

Persons operating pitfall-trap arrays should be trained to identify all focal species that are likely to be trapped in the study area and should be aware of other species that may be trapped incidentally. This can be done by studying field guides, museum specimens, and/or by training with an experienced field biologist. It may be helpful to carry field identification guides while sampling. If a field technician cannot positively identify an animal, he or she should photograph it or return it to camp for further examination. The field crew should also be trained and make an effort to identify the sex, relative age (juvenile/adult), and reproductive condition of a trapped animal (see Chapter 10, "Determining Age, Sex, and Reproductive Condition").

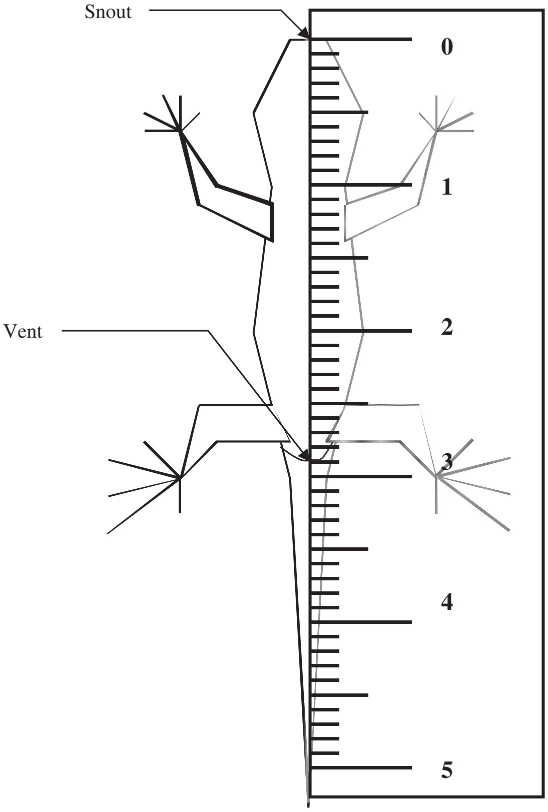


FIGURE 85 Measuring snout-to-vent length (SVL). A ruler is pressed against the underside of the animal, aligned with the end of the snout, and read at the vent, in millimeters. The lizard in the diagram has an SVL of 29 mm.

PROCESSING

The first step when processing an animal is to record the identification code of the array, arm, and trap in which it was caught, followed by its species identification; sex, age, and reproductive status; and unusual markings, deformities, and/or injuries. Next, the investigator measures the animal's length (tip of the snout to the vent, on the ventral side) in mm, with a ruler (Fig. 85). A longer measuring tape is needed for most adult snakes and other large reptiles. Animals are weighed in bags with a spring scale that has been zeroed to accommodate the tare of the weigh bag. Weight is recorded as accurately as the spring scale displays. For safety reasons, we do not measure or weigh venomous snakes; length, age, and sex can be approximated without handling.

To study various demographic parameters as well as animal movements, investigators must be able to recognize individual animals. Color marks can be painted on the scales or shells, depending on the level of permanency needed in the study (Cagle 1939; Woodbury 1953). A common alternative is to clip toes, scales, or scutes, although some limbed animals with certain anatomical traits are unsuited for toe clipping (Fisher et al. 2008). Clipping follows a predetermined number system such that no two individuals of a species at a study site have the same number (Fig. 86). Each captured individual (except venomous snakes) is inspected for prior markings. If they are present, then the animal is identified as a "recapture," and its number is recorded. An unmarked animal is assigned a new number in sequence. The number is then marked off or otherwise identified as used. Numerous numbering systems are available (see Chapter 9, "Marking Reptiles"); we use the one in Fisher et al. (2008). Whatever numbering scheme is used must be well documented in project notes, publications, and associated metadata.

Most lizards and crocodilians are toe clipped for identification purposes. The toes are clipped according to a numeric scheme, except that the accelerator toes (the long toes) on the hind feet of lizards are not clipped. On occasion, animals may be missing a toe(s) due to natural causes; often, a number that incorporates or corresponds to the missing toe(s) can be assigned to an animal. Toes should be clipped with very sharp scissors at the distal knuckle. If additional tissue is required for genetic work, 5 to 10 mm of tail tip (of tailed specimens) can also be clipped. Applying pressure to the wound reduces bleeding. Submersing the animal in a jar of sand is also effective (P. Medica, pers. comm.). Chelonians can be individually marked by making notches on the edges of the marginal scutes, by painting the shells, injecting PIT tags (see "Permanent and Temporary Tags," in Chapter 9, "Marking Reptiles"), or by attaching tags with wires passing through holes drilled in the shell. Investigators planning to mark the scutes should contact other researchers who have previously or are presently studying the species in the same area to coordinate numbering schemes and share data for already-marked individuals. This is more important in chelonians than in most other reptiles because of their longevity and the possibility of multiple individuals studying a particular population over several decades. Different researchers have used angle files or scissors to mark small chelonians, but a hacksaw blade or

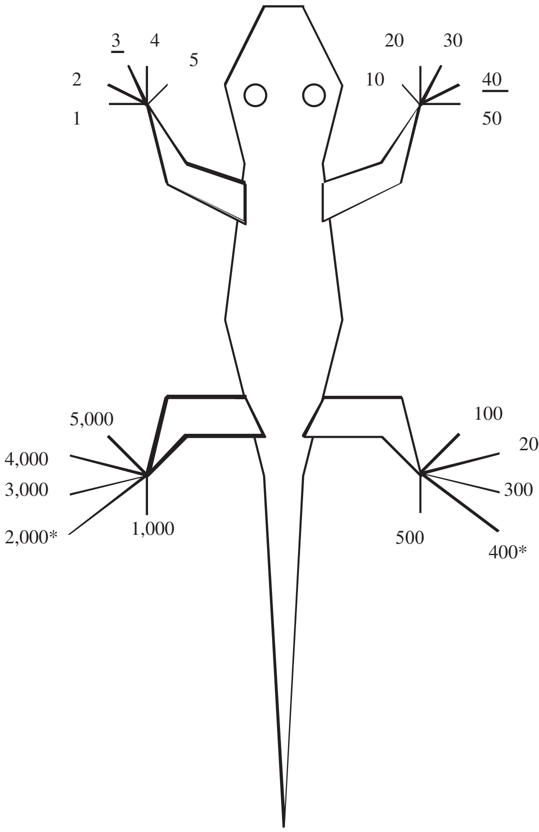


FIGURE 86 Toe-clip diagram. Reptiles with arms and legs are usually marked by toe-clipping. Each toe is assigned a value, starting with the outer toe of the front left limb and proceeding in a clockwise pattern. Toes on the front left limb number from 1 to 5, on the front right limb, from 10 to 50, on the right hind limb, from 100 to 500, and on the left hind limb, from 1,000 to 5,000. For the lizard illustrated, number 0043, toe "40" on the right forelimb and toe "3" on the left forelimb would be clipped. We do not recommend clipping toes "400" and "2,000" on the hindlimbs, because they are important in lizard locomotion.

grinder will be needed for marking larger ones (Woodbury 1953). For chelonians, the tail tip is the only tissue sampled. Because many chelonians are endangered or threatened, we have taken measures to reduce the capture of species that are not being studied (Fisher et al. 2008).

Most snakes are marked by clipping their post-anal (or ventral) scales according to a predetermined numbering scheme. Venomous snakes (e.g., Viperidae), blind snakes (e.g., Typhlopidae, Leptotyphlopidae), and legless lizards (e.g., Anniellidae) are typically not marked. The post-anal scales are located on the ventral side of a snake's tail, posterior to the vent or anal plate, and, depending on the species, are divided or undivided. We assign numbers by looking at the individual from the ventral side with the head at 12 o'clock. For snakes with divided post-anal scales, the two columns of scales are numbered 1 to 9 beginning with the scales closest to the vent and working toward the end of the tail. The left side represents the hundreds (100–900), and the right side represents the tens (10–90). Snakes with undivided post-anal scales are treated like snakes with divided scales. An imaginary line separates a scale into left and right halves, which are clipped in the same manner as if they were separate scales. To reduce the chance of infection near the vent area, the first "two" scales on each side generally are not clipped. Again, various acceptable methods of marking snakes exist (see Chapter 9, "Marking Reptiles"). The important thing is to clearly document the method used in the protocol to prevent confusion among others looking at the data, preserved specimens, or recaptured individuals later on.

Small sharp scissors are used to clip the outer corners of scales. It is important to clip deep enough into the scute so that it does not regenerate. Extra scales on the belly or 5 to 10 mm of tail tip can be clipped from medium and large snakes for additional tissue. Five to 10 mm of tail tip can be taken from animals such as small snakes and limbless lizards whose scales are too small to clip.

Clipped scales, tail tip, and/or other tissues are collected, preserved for genetic analysis, and labeled according to methods outlined in "Collecting and Preserving Tissues for Biochemical Analyses," in Chapter 7. Following processing, the animal is released into nearby vegetation or other cover.

INCIDENTAL CAPTURES AND OBSERVATIONS

Many nontarget species are incidentally trapped or observed. How these animals are recorded and processed depends on the objectives of the research project. If of no relevance, live animals are released; dead ones can be collected and prepared as specimens if the appropriate permits are in hand. If investigators intend to collect incidental specimens, they should have tools and materials necessary for their preparation available.

Pitfall Capture Data

The majority of the data recorded for each captured animal is the same whether documented on paper or on a handheld computer (Figs. 87 and 88). Below we provide a list of standard data fields and their definitions.

Date: date the sample is taken. On a handheld computer (HC), this field is automatically generated based on the HC's preset, internal clock.

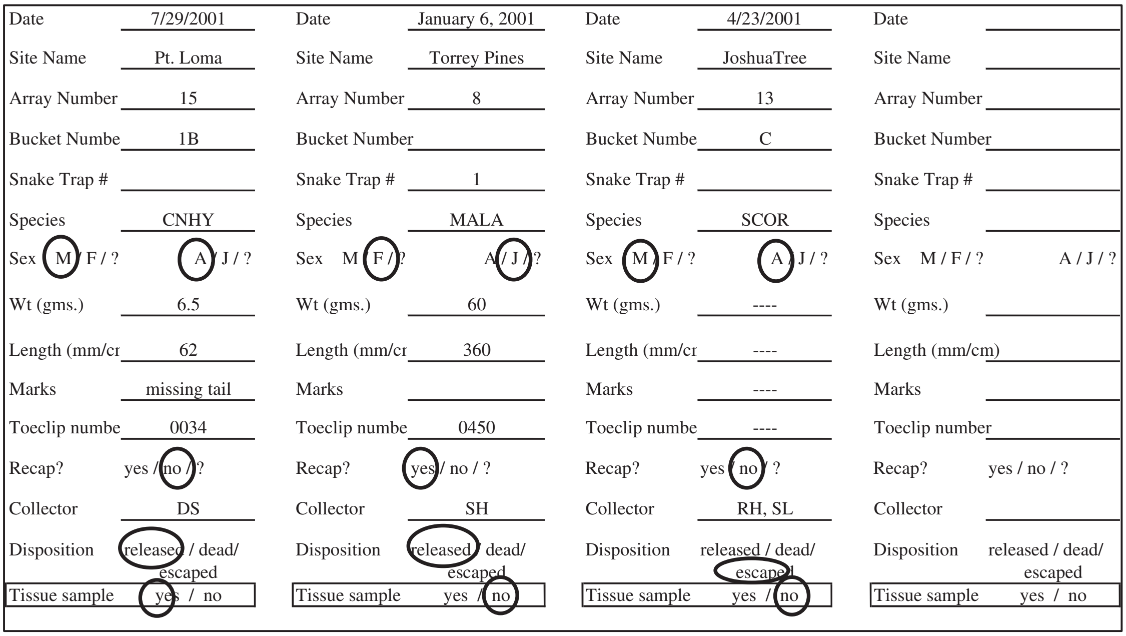
Site name: overall study area and its components. On an HC, the site name is entered once, at the start of the fieldwork; it is automatically carried over to subsequent records, until the collector changes the site name.

Array number: individual identification number for each multiple drift-fence/pitfall/funnel-trap study plot within a study site.

Bucket number: unique, preassigned identifier based on the array and arm numbers and the bucket's position along the arm (see Fig. 75). On an HC, the collector selects the bucket number from a predefined list.

Snake-trap number: unique, preassigned identifier based on the numbers of the array and arm where the funnel/snake trap is located. On an HC, the numbers appear on a pop-up list.

Species: four-letter code consisting of the first two letters of both the genus and species names. For example, SCOC would designate the Western Fence Lizard (*Sceloporus occidentalis*) and RHLE, the Long-nosed Snake (*Rhinocheilus lecontei*). On an HC, names and codes appear on a



Date	7/29/2001	Date	January 6, 2001	Date	4/23/2001	Date	
Site Name	Pt. Loma	Site Name	Torrey Pines	Site Name	JoshuaTree	Site Name	
Array Number	15	Array Number	8	Array Number	13	Array Number	
Bucket Numbe	1B	Bucket Number		Bucket Numbe	C	Bucket Number	
Snake Trap #		Snake Trap #	1	Snake Trap #		Snake Trap #	
Species	CNHY	Species	MALA	Species	SCOR	Species	
Sex	[✓ M] / F / ? [✓ A] / J / ?	Sex	M / [✓ F] / ? A / [✓ J] / ?	Sex	[✓ M] / F / ? [✓ A] / J / ?	Sex	M / F / ? A / J / ?
Wt (gms.)	6.5	Wt (gms.)	60	Wt (gms.)	----	Wt (gms.)	
Length (mm/cr	62	Length (mm/cr	360	Length (mm/cr	----	Length (mm/cm)	
Marks	missing tail	Marks		Marks	----	Marks	
Toeclip numbe	0034	Toeclip numbe	0450	Toeclip numbe	----	Toeclip number	
Recap?	yes / [✓ no] / ?	Recap?	[✓ yes] / no / ?	Recap?	yes / [✓ no] / ?	Recap?	yes / no / ?
Collector	DS	Collector	SH	Collector	RH, SL	Collector	
Disposition	[✓ released] / dead/ escaped	Disposition	[✓ released] / dead/ escaped	Disposition	released / dead/ [✓ escaped]	Disposition	released / dead/ escaped
Tissue sample	[✓ yes] / no	Tissue sample	yes / [✓ no]	Tissue sample	yes / [✓ no]	Tissue sample	yes / no

FIGURE 87 Paper form for capture data. A sample data sheet showing the data fields for recording information on animals captured at a trap array, with typical data.

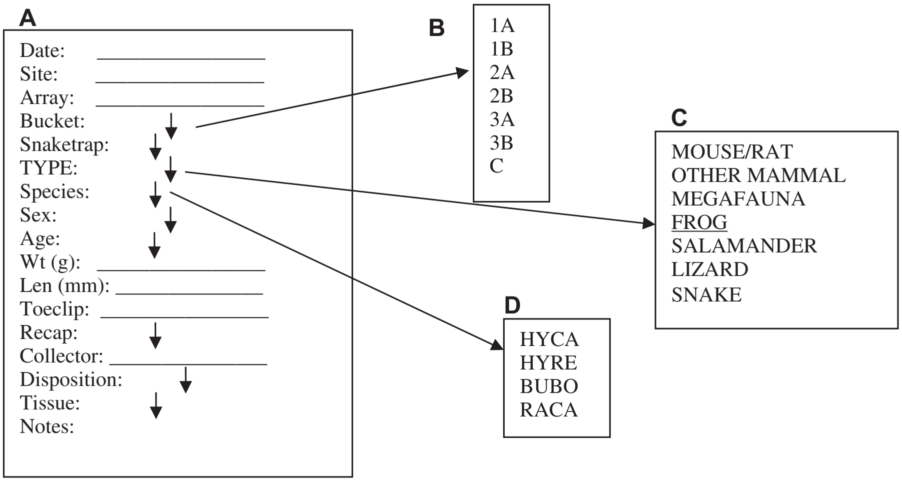


FIGURE 88 Handheld-computer form for capture data. (A) Representation of the data form into which the handheld-computer user enters animal records. (B) Pop-up list of trap numbers appears when the user taps the arrow next to the "Bucket" data field. If the user selects the "Snaketrap" field, a pop-up list of trap numbers appears. (C) The animal "Type" field is selected next. (D) A "Species" pop-up list is displayed when the user taps on the arrow next to the "Species" data field. The pop-up species list that appears is determined by the user's choice in the "Type" data field.

pop-up list; in addition, the species code is augmented by an additional data field (described under "Type," below).

Sex: M/F/? (male, female, unknown) as determined by physical characteristics. On an HC, the designations appear as a pop-up list.

Age: A/J/? (adult, juvenile, unknown) which can usually be established by size and appearance. On an HC, the designations appear as a pop-up list.

Weight: in grams, to the accuracy of the spring scale reading.

Length: measured from snout to vent in lizards, crocodilians, and snakes; carapace length for turtles and tortoises; in millimeters.

Notes: Unusual markings, injuries, deformities, and reproductive status are recorded in this data field. This field is also used to document any outstanding features of the specimen, such as a lost tail or degree of regeneration in lizards, scars, or missing toes. Commonly used phrases appear as a pop-up list on an HC.

Toe-clip number: four-digit figure, for consistency, even if only one toe on one foot has been clipped. Number 5 would be 0005. Each site has its own, independent set of toe-clip or shell-mark numbers.

Recap: Y/N/? (yes, no, unknown). Each recapture must be carefully evaluated because animals can lose their toes naturally.

Collector: typically, the initials of the observer(s) or surrogates for them in the case of duplication. Keys to the initials, with full names, should be maintained in the laboratory and with the data themselves.

Disposition: R/D/E (released, dead, escaped). Most animals are freed following processing. Weights and lengths of dead animals in the traps are recorded and the body saved for a voucher and tissue sample. If a specimen is in poor condition, it may be discarded. Animal escapes should be noted to explain incomplete data.

Tissue: Y/N (yes, no). Whether or not a tissue sample was collected as the animal was processed. Tissue is taken from recaptured animals only if it has not been collected previously.

Type: On an HC, a pop-up list that identifies the kind of animal (e.g., snake, turtle, etc.) being processed (Fig. 88C). When this data field is used, the species designators that appear on the pop-up list pertain only to that group.

Accuracy and completeness of the data collected should always be reviewed by the field technician immediately after returning from the field while the captures from the day are still fresh in his/her mind. The data are reviewed a second time when they are entered into a spreadsheet to ensure that they accurately mirror the data collected from the field (see “Data Quality Assurance and Quality Control,” in Chapter 4).

Data Organization

We store all the data collected on a desktop computer within folders specific to each site or in a single relational database. Site folders contain subfolders with spreadsheet files on the reptiles recorded at the site; small mammals that we documented; charts of the marking schemes (e.g., toe-clip charts) used for the animals at the site; a weather folder; a map folder; a vegetation folder, which contains the vegetation survey data for each array; and folders for other ancillary information. Among the site maps is a topographic map of the study site showing the general locations of the arrays. This is generated using a topographic mapping program or other geographic information system program. Other metadata important to maintain include the latitude and longitude of each array, the sampling history of the site, the number of days the site has been sampled, and a brief description of the site.

Data Analysis

Data generated from our survey methods can be used to address a variety of conservation and management questions. For example, species lists for a study site can be compared to historical records from the same site, if available. Such comparisons can document local and regional species declines as well as range expansions (Shaffer et al. 1998). They bring attention to species that may be particularly vulnerable to human disturbance or habitat fragmentation and thereby stimulate further research, monitoring, and protection for those species; they also form a basis for making many land- and resource-management decisions.

Using capture rate and mark-recapture data, the relative abundances of species can be calculated between arrays within a study site or among study sites and seasons. For a within-site comparison, the total number of first captures (animals recaptured within each sample period are removed) is divided by the number of sample days over which the samples were collected. This produces an average capture rate per day for each species at each array and across the site as a whole. In this example, the data are averaged across sample periods, seasons, and years. Because we used the same sampling technique consistently at all study sites, we can also compare capture rates between sites, but the number of arrays at the sites being compared must be considered. For example, our Point Loma site includes 17 study arrays, at Mission Trails Regional Park we have 5, and at the San Diego Wild Animal Park, 20. To correct for this, the total capture rate per species per day is divided by the number of arrays at the study site.

The data collected during a survey can also provide important information on the demographic and life history characteristics of species, including their reproductive cycles, age-class characteristics, survivorship, species interactions, and habitat associations (Fisher et al. 2002). Seasonal activity patterns of different age and size classes can be determined using an analysis of length or weight versus time. Also, correlations between such factors as patch size, distance from edge, and percent nonnative vegetation may be useful for assessment of human impacts on species abundance and distribution.

For reviewing and summarizing survey data, the Microsoft Excel “Table” option is useful. “Species by Array” tables can be generated to count the number of times that each species has been documented at each array, as can “Species by Sample Period” (or multiple sample periods) tables. They data can also be manipulated to reflect specific conditions in any of the data fields, such as removing and counting the number of recaptures. Other analyses can be performed on the survey data, depending on the data collected and the research objectives (see review in Fisher et al. 2008). Most importantly, it is essential, before beginning a project, to ensure that the data to be collected during the study will be appropriate and sufficient to answer the questions being asked.

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Sampling with Artificial Cover

J. Steve Godley

Artificial cover objects (ACOs) are items set out in a habitat for the purpose of sampling animals that may shelter beneath them. ACOs can be made of a variety of materials, including wood, tin, Masonite, concrete, and asphalt roofing material, and are easily deployed flush with the ground or slightly

TABLE 22

Summary of Artificial Cover Object Literature

Study	*Location: Country/State*[a]	*Habitat(s)*	*Survey Purpose*	*ACO Type(s)*[b]	*Other Survey Methods*[c]	*Relative Sampling Efficiency*
Braithwaite et al. 1989	UK	*Calluna* heathland	Natural history of *Coronella austiaca*	T	VES	ACO>VES
Fitch 1992	USA/KS	Woodland, old field	Effects of sampling method	T, W	DF/FT, VES, RS	ACO>DF>VES, but varied by species
Grant et al. 1992	USA/SC	Pine plantation, upland forest, Carolina Bays	Biodiversity, habitats, methods, ACO type and age	W, T	DF/PFT	DF>ACO
Schlesinger and Shine 1994b	AUS/NSW	Experimental rock crevices in lab	ACO microhabitat selection in *Oedura lesueurii*	S		
Parmelee and Fitch 1995	USA/KS	Woodland, old field	Effects of sampling method	T, W		T = W
Barker and Hobson 1996	UK	*Calluna* heathland	Natural history of *Coronella austiaca*	RT	VES	VES>ACO
Perison et al. 1997	USA/SC	Bottomland hardwood swamp	Compare timber harvest techniques	W	DF/PFT	
Reading 1997	UK	*Calluna* heathland	Biodiversity, effects of sampling method	T	VES	ACO>VES
Downes and Shine 1998a	AUS/NSW	Experimental rock crevices in lab	Snake–lizard prey coevolution	S		
Downes and Shine 1998b	AUS/NSW	Experimental rock crevices in lab	Effects of ACO thermal properties and social dominance on snake–lizard prey coevolution	S		
Adams et al. 1999	USA/WA	Douglas fir forest	Biodiversity	W, PS	VES, FT	VES>ACO, PS>W
Bonnet et al. 1999	France	Parkland	Dispersal mortality	C	VES	
Leiden et al. 1999	USA/SC	Pine plantation, upland forest, Carolina Bays, bottomland hardwoods	Methods, biodiversity	W	DF/PFT, FT, VES	
Russell and Hanlin 1999	USA/SC	Isolated wetlands and adjacent uplands	*Carphophis amoenus* habitat	W	DF/PFT	DF>ACO
Sutton et al. 1999	USA/FL	Scrub	Methods, habitats for *Plestiodon reynoldsi*	W	DF/PFT, E	ACO>DF
Engelstoft and Ovaska 2000	Canada/BC	Douglas fir forest	Biodiversity, methods	R, T, W		R>T>W
Hoyer and Stewart 2000	USA/CA	Rock outcrops in scrub, grassland and forest	Natural history of *Charina bottae*	W, CA	VES	ACO>VES
Webb and Shine 2000	AUS/NSW	Sandstone outcrops	ACOs for habitat restoration	C		
Drost et al. 2001	USA/AZ	Short grass prairie	Biodiversity	W	DF/PFT, RS, VES	ACO>DF, RS>VES>ACO

Jenkins et al. 2001	USA/TN	Old field	Biodiversity, natural history	T, W	VES	T = W, ACO>VES
Faccio 2001	USA/VT	Hardwood forest, conifer plantations	Biodiversity	W	DF/PFT, VES, FT	VES>ACO = DF
Kjoss and Litvaitis 2001	USA/NH	Forest, farmland and developed	Biodiversity, effects of sampling methods	PS	DF/FT	ACO>DF, but varied by species
Metts et al. 2001	USA/SC	Beaver impoundments	Habitats, biodiversity	W	DF/PFT, FT	
Downes and Bauwens 2002	Croatia	Experimental slate ACOs in lab	Interference competition between *Podarcis sicula* and *P. meliscellensis*	SL		
Russell, Guynn, and Hanlin 2002	USA/SC	Isolated wetlands and adjacent uplands	Biodiversity, methods	W	DF/PFT	DF>ACO
Ryan et al. 2002	USA/SC	Pine plantation, upland forest, Carolina Bays, bottomland hardwoods	Biodiversity, methods	W	DF/PFT+FT, VES	DF>ACO> VES
Langkilde and Shine 2004	AUS/NSW	Experimental plastic ACOs in lab	Interference competition for shelters among skink species	P		
Michael et al. 2004	AUS/Victoria	Grasslands and open woodlands	Biodiversity; ACO type, size and age; season	FP		
Shah et al. 2004	AUS/Victoria	Experimental rock crevices in lab	ACO microhabitat selection in *Nephrurus milii*	CT		
Willson and Dorcas 2004a	USA/NC	Mixed hardwood/pine forest	Survey small, fossorial snakes; methods	W	DF/PFT, VES, FT	Varied by species
Ford and Hampton 2005	USA/TX	Prairie woodland	Biodiversity, methods	W, T	VES, FT, RS	VES>ACO, RS>ACO for snakes
Kuhnz et al. 2005	USA/CA	Coastal dune	Effects of sampling method, habitat and soil type on *Anniella pulchra* density	W	VES	VES>ACO
McCauley et al. 2006	Kenya	*Acacia* savannah	Large herbivore effects on snakes and small mammal prey	W	N/A	
Smith et al. 2006	USA/GA	Longleaf pine	Biodiversity	W	DF/PFT and FT, FT, VES, RS	
Wilgers and Horne 2006	USA/KS	Tall grass prairie	Effects of burn frequency and methods on biodiversity	W	DF/FT	ACO>DF
Hampton 2007	USA/TX	Bottomland hardwood forest	Biodiversity, methods	W, T		T>W
Lettink and Cree 2007	New Zealand	Coastal shrubland	Effects of ACO type, season and site	O, C, T	PFT	ACO>PFT for geckos, PFT>ACO for skinks; O>T>C for geckos

(*continued*)

TABLE 22 (*continued*)

Study	*Location: Country/State*[a]	*Habitat(s)*	*Survey Purpose*	*ACO Type(s)*[b]	*Other Survey Methods*[c]	*Relative Sampling Efficiency*
Pike et al. 2007	USA/FL	Native and altered scrub and sandhill	Effects of soil and habitat on *Plestiodon reynoldsi*	W		
Pike, Peterman, Mejeur et al. 2008	USA/FL	Native and altered scrub and sandhill	Effects of sampling method and ACO age on *P. reynoldsi*	W	VES	ACO>VES
Pike, Peterman, and Exum 2008	USA/FL	Native and altered scrub and sandhill	Effects of habitat and soil types	W		
Croak et al. 2009	AUS/NSW	Sandstone outcrops	Restoration potential and colonization rates of ACOs	C		

a. Country/State: AUS=Australia, AZ=Arizona, BC=British Columbia, CA=California, CO=Colorado, FL=Florida, GA=Georgia, IA=Iowa; KS=Kansas, MI=Michigan, NC=North Carolina, NH=New Hampshire, NSW=New South Wales, SC=South Carolina, TN=Tennessee, TX=Texas, USA=United States of America, VT=Vermont, WA=Washington.

b. ACO construction materials: C=concrete, CA=carpet, CT=ceramic tiles, FP=fence posts, O=Onduline, P=plastic, PS=plastic sheet, R=asphalt roofing material, RT=roofing tile, S=sandstone, SL=slate, T=tin, W=Wood.

c. Other survey methods: DF=drift fence, E=enclosure, FT=funnel trap, PFT=pitfall trap, RS=road survey, VES=visual encounter survey.

above it. They can be placed in the environment in standardized experimental arrays to determine the distribution and abundance of individual species or species groups and to identify factors that may affect those traits. When coupled with other sampling methods, such as enclosures, drift fences, and mark-recapture, indices of population size can be developed and used to monitor species through time or space. In recent years, the use of ACOs to inventory and monitor herpetofaunal populations has become increasingly common. Although experimental work employing the technique has focused on terrestrial amphibians (literature summarized in Fellers and Drost 1994; Houze and Chandler 2002; and Marsh and Goicochea 2003), the ACO techniques used also are applicable to many reptilian species.

Target Organisms and Habitats

Reptiles and the habitats they occupy are highly diverse and yet often poorly known. Thus they are excellent targets for inventory and monitoring studies and provide numerous options for experimental work. In Table 22 I have summarized studies of reptiles that involved the use of ACOs to illustrate the range of target species with which they have been used, the range of habitats occupied by those species, and the variety of questions addressed. I have included only those studies that were conducted for at least one complete activity season (or major lab experiment), differentiated between reptile and amphibian observations, and included more than 500 ACO trap checks. I also reviewed approximately 50 additional publications or studies that do not meet these criteria.

Perhaps because of my biases and those of the major search engines, most (25) of the 41 publications cited in Table 22 were conducted in the United States, followed by Australia (8), the United Kingdom (3), and five other countries (1 each). Most field studies were conducted in temperate, forested, upland environments (31), although dry grasslands and deserts were often sampled also (10). No studies were carried out in tropical forest, possibly because above-ground roots prevent ACOs from lying flat on the ground (Doan 2003; but see Sawaya et al. 2008). In six of the studies, investigators used ACOs in the laboratory to substantiate field observations, typically by experimentally exploring microhabitat selection, competition, or predator–prey coevolution. Several investigators not cited in Table 22 manipulated natural cover objects, often in elegant experimental designs, to evaluate retreat site selection (Huey et al. 1989; Kearney 2002; Sabo 2003) or the consequences of rock habitat degradation on lizard and/or snake populations (Schlesinger and Shine 1994a; Goldingay and Newell 2000).

Overall, ACOs most effectively sample snakes and lizards that are small relative to the size of the cover objects, species that seek cover beneath surface objects in the landscape, and habitats where natural cover is limited and/or the ACOs can be easily sampled and deployed.

Background

Many species of reptiles use natural surface objects as resting or foraging sites, as cover where they can hide from predators or thermoregulate, and/or as places where they can deposit their eggs or brood their embryos. The use of such objects may vary seasonally or even on a daily basis in some species (Grant et al. 1992; Jenkins et al. 2001). Herpetologists have long capitalized on such behavior by systemically searching under natural cover objects as a means to inventory animal populations in specific habitats. By employing time- or area-constrained transect counts or quadrant searches, investigators can estimate relative abundance and species diversity (Campbell and Christman 1982; Vogt and Hine 1982; Corn and Bury 1990; Heyer et al. 1994a).

Nevertheless, searching under natural cover objects has a number of drawbacks. Typically, the cover object and the habitat around it are destroyed, making repeated searches of the same site or stations impossible and increasing the vulnerability of the animals to environmental threats. Such searches can also injure or kill the study (or other) organisms. In addition, they are difficult to standardize because the number, size, and distribution of natural surface objects often vary among sites, and because between-observer biases are often considerable.

The use of ACOs eliminates many of those difficulties while offering a number of advantages. For example, ACOs can potentially yield large samples, although the capture success rate typically varies by species and often depends on weather conditions and season. ACOs can be placed along transects or in plots with a relatively low investments of time and money, can be deployed and checked with little training, are easy to maintain, and can be replicated in various experimental designs through time and space (Grant et al. 1992; Fellers and Drost 1994; Reading 1997; Sutton et al. 1999; Monti et al. 2000). Nevertheless, because ACOs only provide an index of population size or species occurrence, they must be used in conjunction with other sampling and monitoring techniques such as mark-recapture or site-occupancy models to estimate the density or the detectaibility of the species (Mazerolle et al. 2007; see also "Permanent Plots with Mark-Recapture," above, and "Key Definitions," in Chapter 15).

Research Design

Research designs using ACOs often vary depending on the species of interest and the research objectives. The ACOs used in the field studies were most commonly made of wood ($N=26$, typically plywood), followed by tin (10), with other materials used much less frequently (Table 22). Usually investigators cut standard sheets of plywood (12 studies) into four (~61 × 122 cm) or eight (~30 × 61 cm) equal-size pieces (6 studies); most pieces of tin were ~61 × 122 cm. Typically, ACOs were set singly and flush to the ground. However, Lettink and Cree (2007) found that three small pieces of corrugated onduline (a type of roofing material) and tin stacked with spacers were effective for sampling gecko species in New Zealand, and several investigators studying lizards and snakes in Australia have used spacers to create artificial crevices above the substrate (Schlesinger and Shine 1994b; Downes and Shine 1998a, 1998b; Webb and Shine 2000; Croak et al. 2010).

In 12 of the cited field studies, investigators used multiple types of ACOs in paired trials; a few of them compared the sampling efficiencies (capture success) of the ACO types for reptiles, often with conflicting results (Table 22). In these comparative studies, thermal regimes exerted a strong effect on ACO use. Reptiles in cool, temperate climates chose dark-colored, heat-absorbing ACOs over other types (Barker and Hobson 1996; Adams et al. 1999; Engelstoft and Ovaska 2000;

Lettink and Cree 2007). Time of day, season, canopy closure, and moisture levels also influenced relative capture success in these studies and others (Grant et al. 1992; Parmelee and Fitch 1995; Jenkins et al. 2001; Michael et al. 2004; Hampton 2007). In general, the use of multiple types of ACOs increased the number of species and individuals of reptiles captured.

Most investigators sampling reptiles deployed ACOs in conjunction with other survey methods, particularly if the goal was to evaluate local biodiversity or relative habitat use (Table 22). ACOs have been paired most frequently (Table 22) with drift fences set with pitfall traps and/or funnel traps (see "Pitfall-Trap Surveys," above), various forms of visual encounter surveys (see "Visual Encounter Surveys," above), and/or road surveys (see "Road Running," above).

No standardized research design emerged from these studies, although most replicated the same design at each sampling plot. In three studies (Grant et al. 1992; Russell and Hanlin 1999; Russell, Guynn, and Hanlin 2002), investigators encircled isolated wetlands with drift fence/pitfall-trap arrays and then used linear transects of ACOs to sample adjacent uplands. Several other investigators coupled drift fence/pitfall-trap arrays of varying designs with adjacent, radiating transects of ACOs (Drost et al. 2001; Wilgers and Horne 2006). Reading (1997) proposed hexagonal arrays of evenly spaced tin ACOs as a standard method for surveying reptiles in the United Kingdom.

Estimates of the relative sampling efficiencies of different survey methods are shown in Table 22 for studies in which ACOs were used and effort was quantified. For this analysis, the number of reptiles recorded per total number of ACO check days was compared with the number of reptiles captured per total drift-fence/pitfall-trap days of effort. Using this metric, drift fence/pitfall traps outperformed ACOs in four studies conducted in South Carolina forests (Grant et al. 1992; Perison et al. 1997; Russell and Hanlin 1999; Ryan et al. 2002), but ACOs were generally superior in prairie habitats (Fitch 1992; Drost et al. 2001; Wilgers and Horne 2006), for most snake species in New Hampshire (Kjoss and Litvaitis 2001), and for the Florida Sand Skink (*Plestiodon* [= *Neoseps*] *reynoldsi*; Sutton et al. 1999). In several studies, investigators compared the sampling efficiency of visual encounter surveys (see "Visual Encounter Surveys," above) made by counting the number of reptiles observed while walking between ACOs or per man-hour of search effort with that of the ACOs. Again, results were mixed, depending on the species and habitat (Table 22).

Some evidence suggests that the use of ACOs by reptiles increases with time in the environment and that ACOs can increase the carrying capacity of a habitat. Grant et al. (1992), working in South Carolina found significantly more reptiles beneath 1-y-old tin sheets than under newly placed (≤2 months) tin ACOs. In Kansas, 7-y-old wood and tin ACOs yielded 20 percent more rodent-eating snakes (but fewer smaller species) than new ACOs paired with them. In Australia, significantly more individuals of two lizard species and an elapid snake (*Suta suta*) were captured under fence posts that had lain *in situ* for more than 15 years than under newly placed posts of similar size (Michael et al. 2004). These authors suggested that *S. suta* preferentially used the abundant invertebrate burrows beneath old fence posts as retreat sites and increasingly used new posts as the number of invertebrate burrows and prey (lizards and mammals) increased. Using artificial rocks in Australia, Croak et al. (2009) quantified the sequential rates of colonization by 45 species of invertebrates, six species of predatory lizards, and two species of snakes that prey on the lizards. Over the 40-week study period, the number of species of lizards reached an asymptote at week 32 and the numbers of invertebrates and snakes at week 36. By the end of the study, all of the artificial rocks were used by fauna, with 99.5 percent occupied by invertebrates and 81.8 percent occupied by reptiles; however, the total number of individually marked lizards continued to increase with time.

Sutton et al. (1999) is the only study to directly link ACO use with the actual abundance of a reptile species (Florida Sand Skink) in the field. This small, fossorial lizard is endemic to xeric upland habitats (see descriptions of sandhill, scrub, and scrubby flatwoods in Abrahamson and Hartnett 1990 and Myers 1990), where it "swims" through the sand, leaving distinct, sinusoidal tracks (for track photographs, see Pike, Peterman, and Exum 2008). Although wind and rain quickly obliterate these tracks, they do not degrade for at least 1 week beneath plywood ACOs (JSG, pers. observ.). Using mark-recapture techniques, Sutton et al. (1999) determined total skink abundance within 20-×20-m enclosures, each set with a drift fence and pitfall traps and four ACOs. They demonstrated that total abundance was strongly correlated with the abundance of tracks under the coverboards ($r_s = 0.82$, $P < 0.05$, $N = 7$) at skink densities ranging from 125 to 650 individuals per ha. ACOs set in standardized arrays and checked weekly during peak activity periods have also been used to detect the occurrence and habitat distribution of this Florida and federally threatened species (Sutton et al. 1999; Pike et al. 2007; Pike, Peterman, and Exum 2008; Pike, Peterman, Mejeur et al. 2008).

Field Methods

Depending on research objectives, ACOs can be randomly scattered throughout the habitat, strategically placed where capture success is judged likely to be highest, or set in standardized grids or transects. Likewise, the size, type, and density of ACOs as well as the frequency, length, and timing of ACO trap checks may vary. To date, only a few studies have evaluated the relative efficiencies of these different sampling regimes for reptile species (Grant et al. 1992; Parmelee and Fitch 1995; Adams et al. 1999; Sutton et al. 1999; Engelstoft and Ovaska 2000; Jenkins et al. 2001; Hampton 2007; Lettink and Cree 2007). Projects employing ACOs would benefit from pilot studies to judge the effectiveness of different ACO placements, particularly if the aim of the study is to estimate biodiversity or the presence of particular species (Mazerolle et al. 2007). In the absence of such information, an investigator should adhere to the following general guidelines:

1. Ensure that the size, type, and sampling density of the ACOs are consistent with the body size(s) and estimated home range size(s) of the study organism(s).
2. Use ACOs made of as many different materials (especially wood and tin) as possible, depending on research objectives.
3. Use standardized grids or transects with replicates to increase the statistical robustness of the experimental design.
4. Sample during all activity seasons and at all times of the day.
5. Quantify all relevant environmental variables (e.g., temperature, moisture, and weather conditions), ambient as well as beneath the ACOs.

6. Check sites at times and under weather conditions as similar as possible, if encounter rates are to be compared among sites.
7. Sample individual ACOs at least once per week.
8. Recognize that encounter rates may vary in long-term studies with the time since ACO deployment, and that individuals of some species may return repeatedly to the same ACO.
9. Mark all captured individuals and note recaptures.
10. Use ACOs in concert with other sampling techniques (e.g., enclosures and/or drift fence/pitfall arrays) if population indices are to be developed.
11. Quantify the sampling effort of all methods used, if capture efficiency of ACOs is to be compared with those of other survey methods.

In the field, ACOs typically are placed flush with the ground, which may require the removal of rocks, logs, and other debris. Placing sand beneath the ACO will facilitate recording tracks of animals such as Florida Sand Skinks. Plywood less than 0.67 cm thick often warps badly in the field, particleboard disintegrates quickly, and both are susceptible to termite damage (JSG, pers. observ.). An intact roll of asphalt roofing material is easier to carry into the field than rigid ACOs and apparently does not release contaminants into the environment (Minahan et al. 2002). Investigators can check an ACO by quickly lifting it and capturing all individuals beneath. Captured individuals can be held temporarily in plastic or cloth bags, but processing (i.e., mark, measure, weigh) them at their capture sites is better because it will minimize negative effects on behavior. The investigator also records the date and time of capture and then releases the animal either underneath or immediately adjacent to the ACO (be consistent). Once checked, an ACO should be returned to its original position. Finally, wearing gloves or other protective gear is wise if venomous insects and reptiles are common in the study area and congregate under ACOs.

Resources

Sampling design and objectives, site logistics, cost (time and money), and other project-specific factors will affect the number, type, and size of ACOs that can be deployed in the field. ACO deployment is time and labor intensive, and investigators should plan accordingly. If site-specific capture information is needed, the top of the ACO can be labeled with indelible ink (e.g., Sharpie) or paint for identification of the unique site, transect/grid, and ACO number. Depending on the distances between ACOs, the research design, and the abundance of the organisms, one or two individuals can generally check an array of 200 ACOs in less than an hour. ACO transects can be checked by one person with experience in identifying local reptiles; with ACOs, there is little or no observer bias in search image (Grant et al. 1992). In addition to ACOs, the field researcher must carry the appropriate holding bags, measuring and marking equipment (see Chapter 9, "Marking Reptiles"), and data forms. The latter should be printed on waterproof paper, and the data recorded with permanent, waterproof ink or lead pencil. If listed (threatened or endangered) species may be captured, then the investigator must obtain appropriate state and/or federal research permits.

Surveying for Florida Sand Skink tracks beneath ACOs (including ACO deployment) takes about seven times as long as checking the ACOs. Sutton et al. (1999) estimated that the cost (materials and labor) of one ACO was less than 20 percent of that for one sand skink pitfall/drift fence. Also, some plywood distributors will cut the ACOs at a reduced cost or for free. Finally, an aluminum L-brace can be attached to the end of the 1-m handle of a whiskbroom. In a few seconds, an investigator can use the brace to lift an ACO and check underneath, and use the broom to sweep away skink tracks, without back strain.

Kjoss and Litvaitis (2001) estimated the cost of a single plastic sheet ACO (1.5 × 3.0 m) to be US$1, yet an equivalent section of drift fence/funnel array was US$15 and required much more time and effort to deploy and dismantle. Drift fence arrays also must be checked almost daily when open to prevent death of any organisms (Fitch 1992; Grant et al. 1992; Drost et al. 2001; Kjoss and Litvaitis 2001; Russell, Guynn, and Hanlin 2002; Wilgers and Horne 2006).

Data Treatment and Interpretation

If a researcher adheres to all or most of the field methods recommended in this section, she or he will obtain a robust set of data that can be analyzed under various statistical scenarios. In most cases, data should be recorded at the level of individual ACOs; combining data later is always an option. Testing for the effects of environmental variables (e.g., temperature, litter depth, moisture, rainfall) on rates of encountering reptiles beneath ACOs typically requires that data be transformed so that the assumptions of parametric regression analysis are not violated. On the other hand, numerous nonparametric alternatives for both paired and linear data are available. Researchers should consult Sokal and Rohlf (1995) and Zar (1999) for information on general statistical analyses, and the literature cited in this chapter for examples of analyses used. Most biodiversity indices assume that no individual is counted more than once (Pielou 1977); to avoid violating this assumption, investigators must mark all captured animals individually or as cohorts.

Finally, although ACOs have been used widely to estimate herpetofaunal biodiversity and record the occurrence of species at particular sites (Table 22), only a few investigators (Sutton et al. 1999; Faccio 2001; McCauley et al. 2006; Pike, Peterman, Mejeur et al. 2008) have used site-occupancy models (Mazerolle et al. 2007) to account for differences in species detectability. Several statistical options for comparing the relative sampling efficiency of different survey methods, including ACOs, can be found in Sutton et al. (1999).

Reptile Sign and Camera Stations

Robert N. Fisher

There are many ways to determine the presence of a species without actually capturing or directly observing it. For example, investigators can record the presence of a reptile species during field surveys through the use of visually detected sign such as burrows, tracks, food items, and scat, as well as with photographs of animals taken by unmanned cameras. The use of sign has been best developed for mammal inventories and monitoring (Wemmer et al. 1996). However, each of the techniques has also been used with reptiles and could be employed more frequently to address ecological, behavioral, and biodiversity-related questions.

Some types of sign have been used for many years in systematic ways; others have been employed only recently and show promise. Desert Tortoises (*Gopherus agassizii*), for example, can be located (and then monitored and/or captured) by locating their burrows (Woodbury and Hardy 1948). Lillywhite (1982) used tracks to distinguish among three species of snakes and determine their road-crossing frequencies in the mountains of southern California. Scat transects have been widely used for many years to monitor species of horned lizards (Turner and Medica 1982; Beauchamp et al. 1998), and Emmons (1989) verified jaguar predation on chelonians through observations of kill sites and scat analysis. Camera stations have been used to monitor the movement and nesting behavior of lizards (Bennett and Hampson 2003) and crocodiles (Thorbjarnarson et al. 2000), as well as to obtain photos of reptiles incidental to work with other classes of animals. High rates of snake predation on nest contents have been recorded by video cameras monitoring bird nests (Morrison and Bolger 2002; Peterson et al. 2004). Below I review roles of visually detected sign and unmanned camera stations in reptile inventorying and monitoring and suggest protocols for standardizing their use.

Track Stations

Researchers often rely on indirect methods of observation to obtain indices of animal abundance because density assessments can be logistically challenging and cost prohibitive. A reptile track can be the first and foremost clue to species occurrence in an area. Taking into account such characteristics as size and substrate, tracks can provide a means of species identification. In addition, the location and environment surrounding a track will provide information about both behavior and habitat preference. Locating tracks can be an efficient technique for locating sand-dwelling reptiles and can furnish information concerning their movement patterns and habitat preferences. Tracking media can be useful for recording the movement of reptiles along corridors (i.e., through roadway crossing structures, such as culverts, drainage pipes, and reptile tunnels) and along dispersal routes. While studying reptiles in the Sahara Desert, Mosauer (1932) determined that tracks left in fine sand could be used to distinguish some species of snakes, as well as the type of locomotion used. Mosauer (1933, 1935) was likewise able to differentiate between methods of locomotion and track patterns for various snake species in the Coachella Valley of California. He provided extensive descriptions of the tracks of the Western Shovel-nosed Snake (*Chionactis occipitalis*) and the Sidewinder (*Crotalus cerastes*). Cowles (1941) used track patterns to determine the winter activities of *Chionactis occipitalis* and other desert reptiles.

Track stations have been widely used to determine abundance and monitor population trends in mammals, following methods developed by Linhart and Knowlton (1975). Track stations are fixed sites that can be repeatedly visited and are generally spaced out along transects or trails. They can vary in size, but typically they are 1-m^2 cleared plots (i.e., litter has been removed) covered in sand or a fine-textured substrate (such as gypsum). When reptiles cross these substrates they leave tracks and sign that record their presence and movement. The material and size of the plots does not matter as long as the tracks can be retained between sampling visits. These stations are raked or sifted after sampling to reset any tracks recorded. Many investigators have attempted to define the relationship between the number of visits to track stations and abundance in mammals in order to validate the accuracy of the method (Hatcher and Shaw 1981; Clark and Campbell 1983; Conner et al. 1983; Woelfl and Woelfl 1997; Sargeant et al. 1998). Many discrepancies still exist, although the methods (i.e., plot size, substrate type, transect length, distance between plots) can be standardized. Several investigators have demonstrated the influence of transect interval, station interval within a transect, station substrate, and number of sampling nights on results (Roughton and Sweeny 1982; Sargeant et al. 1998). Nevertheless track surveys provide effective means of determining geographic distribution, habitat use, and relative abundance of mammalian and reptile species (Lillywhite 1982; Conner et al. 1983; Secor 1994; Sargeant et al. 1998).

Track station transects can be established along dirt roads and wildlife trails throughout a designated study area. For example, five stations could be situated along 1,000-m-long transects at approximately 250-m intervals. Because sampling areas are often irregular, however, some transects may contain more or fewer stations. In addition, individual track stations that are not part of continuous track transects can be placed in areas of limited access or where a complete transect is not feasible. These stations are useful in documenting crossing locations of species at critical choke points or along major roadways. They also can be used at targeted sites for detecting specific species, and in some cases boards have been placed over the loose substrate plots to detect tracks of fossorial species (i.e., *Anniella*).

Gienger et al. (2002) used 1-m^2 sand plots as track stations to assess the effects of canopy cover and forest gaps on lizard activity in southwest Mexico. Stations are typically checked for tracks on from 1 to 5 consecutive mornings (Hatcher and Shaw 1981; Martin and Fagre 1988; Hein and Andelt 1994; Heske 1995). Other researchers have argued, however, that for nocturnal mammalian carnivores 1 night of sampling is sufficient (Roughton and Sweeny 1982; Conner et al. 1983; Woelfl and Woelfl 1997; Sargeant et al. 1998), although much less is known about their effectiveness for reptiles, especially diurnal species. The effectiveness of tracking stations at recording reptiles will vary by species, site, and weather. If an animal visits a station, tracks are identified to species if possible, and the station is cleared (with a rake or similar tool) and resifted (with a colander, depending on substrate type) to reset it between visits. Identifying a track to species may be difficult; in some instances, however, characteristics such as hind foot length, tail drags, and possibly body length are evident, as are special potentially distinguishing characteristics (e.g., form of whiptail feet, width of snake drags; Fig. 89). Knowing which species occur in an area is helpful, especially if a picture library of tracks of those species on different substrates is available for comparison.

Track stations operate best during the summer and fall seasons, when reptile activity is greatest. This schedule can be modified to accommodate seasons of precipitation. In xeric regions, monitoring tracks of most species may be easier in the dry season, when snake trails, either crossing dirt roads or in gypsum along paved roads, are more visible (animals drawn to road for heat). When targeting species in mesic areas, wet-season tracking in areas of soft mud and sand may be preferred. Stations may be operable during other months as well; how-

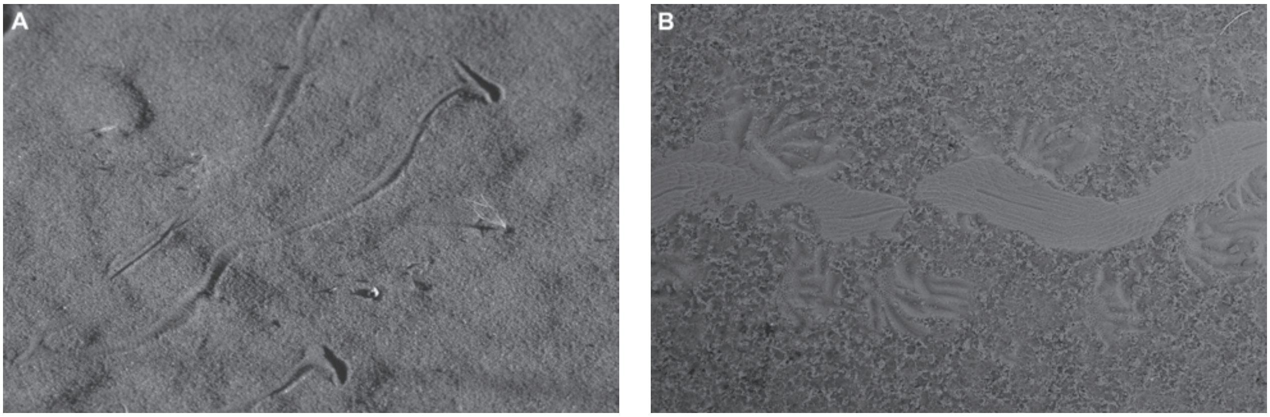

FIGURE 89 Reptile tracks in sand. (A) Sidewinder (*Crotalus cerastes*) in Arizona. (B) Gila Monster (*Heloderma suspectum*) in Nevada. (Photos courtesy of U.S. Geological Survey, Western Ecological Research Center.)

ever, rain during the winter and spring seasons makes track surveys difficult to execute in xeric areas, as gypsum powder is not usable when wet. If stations are to be run during the wet season, it is best to begin surveys 2 to 3 days after a rain.

To obtain an index of animal activity, the number of visits by each species is divided by the total sampling effort. This index is calculated using the following equation:

$$I_j = \{v_j/(s_j n_j)\} \tag{1}$$

where
I_j = index of that species' activity at transect *j,*
v_j = number of stations visited by that species at transect *j,*
s_j = number of stations in transect *j,* and
n_j = number of nights that stations were active in transect *j.*

Any station at which tracks are too difficult to read is omitted from the sample. Thus, the true sampling effort is

$$\{s_j n_j\} - o_j \tag{2}$$

where o_j = number of stations in transect *j* that were omitted.

This index does not provide data on the absolute number of individuals. Instead, it is used to compare relative amounts of species' activity across space and time (Conner et al. 1983; Sargeant et al. 1998). Track indices may be pooled across seasons to derive a single track index per transect for each individual species. These indices serve as the measure of relative abundance in the statistical analysis of the transect data. These types of surveys work well for larger lizards, crocodiles, and turtles, especially if one is trying to identify movement corridors for migration or seasonal habitat use. Depending on the context and the question being asked, they also work well for small lizards and snakes.

A model sampling protocol for a xeric region and the materials required are as follows:

1. Equipment needed: gypsum powder, a colander to sift the gypsum, and a brush to clear an area on the ground (i.e., remove small stones, sticks, and other debris that may interfere with a track outline) so that tracks register clearly.
2. Track stations are established at approximately 250-m intervals along 1,000-m transects located on dirt roads and wildlife trails. A precise 250-m interval is not critical; distances between stations can range from 200 to 300 m (while keeping transect length at around 1,000 m). Station locations should focus on road intersections, drainage crossings, and topographical features. Each transect generally includes five stations, although constraints on sampling area may mean that some transects contain fewer or more stations. In addition, individual track stations that are not part of continuous track transects may be placed in areas where access is limited and/or a complete transect is not feasible. These stations are useful in documenting crossing locations of species at critical choke points or along major roadways or habitat breaks.
3. The 1- × 1-m area selected for the track station, is cleared of all stones, sticks, and other debris, that is, of any material that would cause difficulty in reading a track. Sand or soft dirt can be used as the substrate, or gypsum is sifted through the colander over the cleared area, until it is ca. 1- to 2-cm deep.
4. Track stations are checked for tracks on 5 consecutive mornings. Data can be entered onto a PDA form (see "Handheld Computers for Digital Data Collection," in Chapter 4) or paper data sheets. Typically, the following information is recorded for each *species* that has visited a station: site name, transect name, station number, whether or not there were any visitations (i.e., checked but no tracks, this is important negative data for calculating effort), species identification, and notes (some typical notes are predefined in the form). Only a single entry is made for a species even if it has visited a station more than once (i.e., two sets of lizard tracks enter or leave the station). Thus, a station visited by five individuals representing three different species will have three separate entries in the PDA (one for each species).
5. Once all of the species' visits to a track station are entered, the station is "cleared" by brushing out all of the tracks on or in the vicinity of the station.
6. Managers of some properties may require investigators to clear away any gypsum powder used, when sampling is completed.

7. Typically, track stations are not set if rain is expected in the next 3 days. If it rains before 3 days of sampling are completed, another round of sampling is initiated 2 to 3 days after the rain (so that the ground can dry) and when the probability of having 4 consecutive rain-free days is highest. If it rains after 3 days of sampling are completed, then the round of sampling is considered complete.

We used this protocol in southern California, recording track indexes of 0.237 and 0.441 for snakes and lizards, respectively, in low-elevation culverts, versus 0.011 and 0.093 for snakes and lizards along roads at low elevations or 0.004 and 0.081 at high elevations. Although these data were obtained during studies directed at large mammals, they provide insight into the value of selected culverts for reptile movement under roads in a fragmented landscape (Hathaway et al. 2002, 2004). Lemen and Freeman (1985) tracked small mammals using fluorescent pigments and ultraviolet light in order to obtain very accurate tracking information at fairly low cost (Brehme 2003). It may be possible to do the same with some reptile species.

Visually Detected Sign

STRUCTURES

Various reptiles create structures for shelter or nesting that are easily detected and assigned to species. Lizards and tortoises are responsible for many of the small to medium-size burrows encountered in dry climates. Tortoise burrows are readily identified by their half-moon shape and throw mounds of removed soil at the burrow entrances (Elbroch 2003).

American Alligators (*Alligator mississippiensis*) construct nest mounds of vegetation and mud in which to deposit their eggs. The mounds are often large enough to be located by aerial surveys. Alligator nest mounds can also serve as egg deposition sites for other reptiles, including the Green Anole (*Anolis carolinensis*) and various turtle species (Kushlan and Kushlan 1980).

SIGNS OF FEEDING AND FOOD REMAINS

Reptile remains are often found in the scats or nests of mammalian and avian predators. Predation on Desert Tortoises by Common Ravens (*Corvus corax*) is well documented, and shells of juvenile tortoises are frequently found in or around raven nests (Boarman 2003; Kristan and Boarman 2003). *Anolis* lizards were among the primary prey items (Vilella 1998) in the stomach contents of mongooses (*Herpestes javanicus*) in Puerto Rico.

Bennett and Hampson (2003) mentioned that the bark of many fruit trees on Polillo Island, Philippines, is scratched, a sign of Gray's Monitor (*Varanus olivaceus*) presence and foraging.

SCAT

Scats provide information about reptiles, their diets, and space use within various environments. Reptile scats can be identified to species using characteristics such as size, location, content, and visual attributes, depending on a species' preferred diet and seasonal availability of food. The scat of horned lizards, for example, is readily identifiable because of the harvester ant (*Pogonomyrmex* spp.) exoskeletons it contains, and it is often more visible than the animals themselves. Turner and Medica (1982) and Newbold (2005) used scats to determine distributions and develop relative abundance indices for Flat-tailed Horned Lizards (*Phrynosoma mcallii*) and Desert Horned Lizards (*Phrynosoma platyrhinos*), repectively. Fair and Henke (1997b) used scat counts to determine relative abundance of Texas Horned Lizards (*Phrynosoma cornutum*) in study plots subjected to different land management practices. Scat contents can also be investigated for exact diet composition, information on prey selection (e.g., Suarez et al. 2000), and seasonal shifts in diet (Walls 1981). Reptile scats can contain anything from the undigested chitin of insects to fruit and seeds; data on food source and abundance have contributed to the conservation of habitats for frugivorous species (Bennett and Hampson 2003).

Scats can also be surveyed quantitatively along standardized transects that are surveyed at some given frequency (e.g., daily, weekly, monthly) for scat of specific species that are easily identifiable. Prior to the first sampling bout, all scat observed along the length of the transect is removed. The researcher also determines the distance from the transect that scat can be reliably detected so that sampling effort can be calculated consistently (transect length × transect width; see "Transect Surveys, Including Line Distance," above, in this chapter). During each sampling bout, the investigator follows the transect, recording the scat encountered and removing it to reset the transect for the next survey. Scat transects have been well developed for certain monitoring programs and are used to determine relative abundance or just to determine presence. For reptiles, they are used as part of the Desert Tortoise monitoring and Flat-Tailed Horned Lizard (*Phrynosoma mcallii*) sampling regimes (Turner and Medica 1982; Freilich and LaRue 1998). Eleven species of Australian skinks (*Egernia*) are known to create latrines outside their burrows and dens (Greer 1989; Chapple 2003). Although the significance of these fecal piles remains a mystery, they provide a means to monitor the presence of *Egernia* spp. in an area (Chapple 2003). Chuckwallas (*Sauromalus* sp.) produce similar piles of feces.

Camera Stations

REMOTELY TRIGGERED CAMERAS

Remotely triggered and/or unmanned cameras have become increasingly useful and unobtrusive tools for recording preferences and activities (e.g., habitats used; movement corridors) of various wildlife species, as well as for monitoring them (Griffiths and van Schaik 1993; Jacobson et al. 1997; Karanth and Nichols 1998; Fig. 90). By placing a camera system triggered by a pressure pad at the entrance of a female Gopher Tortoise burrow, one can monitor social behavior (visits, interactions, mounts; Boglioli et al. 2003). Remote cameras have also been used to study the nesting behavior of American Crocodile (*Crocodylus acutus*) in Cuba and Black Caiman (*Melanosuchus niger*) in Brazil, and to identify predators eating Black and Spectacled (*Caiman crocodilus*) caiman eggs (Kermeen and Lemnell 2000). Cameras also provide a relatively low-maintenance means of monitoring and surveying wildlife populations, because the units are visited only to change film (or download images) and batteries.

Several brands of remotely triggered cameras are available commercially. Many camera options are also available because

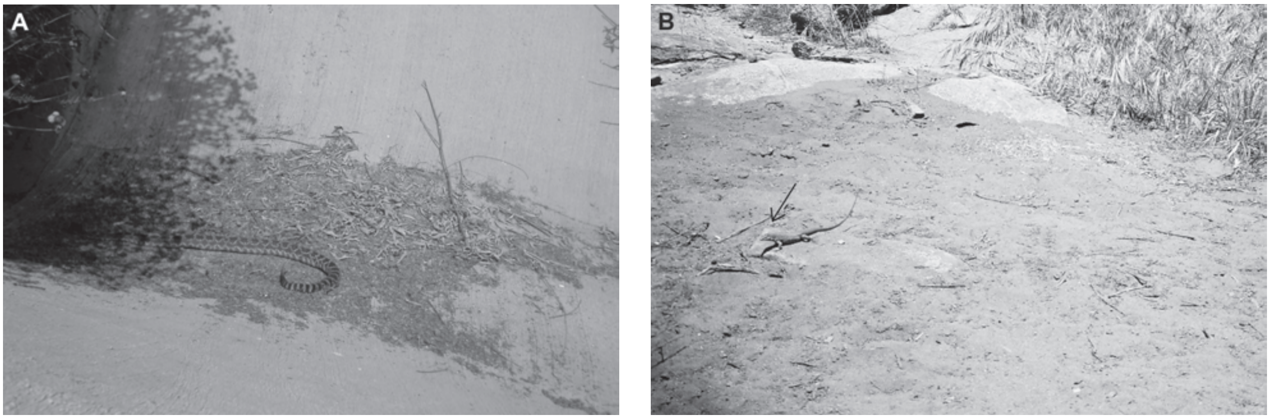

FIGURE 90 Remotely triggered camera detections. (A) Southern Pacific Rattlesnake (*Crotalus oreganus helleri*) using a wildlife underpass. (B) Coastal Whiptail (*Aspidoscelis tigris stejnegeri*) on a trail (see arrow) in southern California. (Photos courtesy of U.S. Geological Survey, Western Ecological Research Center)

the technology is advancing so rapidly. Cameras can be operated any time of year to monitor vertebrate activities. Each pass of an animal by an infra-red sensor or on a pressure plate triggers the camera. Date and, in some instances, time of day are recorded on each print. Camera height and other settings will need to be optimized to the size of the animal(s) of interest to maximize photo captures; cameras work best for larger animals. Van Schaik and Griffiths (1996) examined the activity periods of rain forest animals and the effects of human traffic on wildlife activities using a remote camera system triggered by a pressure plate; they detected both turtles (*Testudo* spp.) and monitor lizards (*Varanus salvator, V. rudicollis*).

To obtain an index of relative activity for each camera, the number of visits recorded for each species is divided by the total sampling effort. This index is calculated using the equation

$$I_j = \{v_j / n_j\} \qquad (3)$$

where

I_j = index of activity for a given animal species at camera *j,*
v_j = number of passes by that species at camera *j,*
n_j = number of nights that camera *j* was active.

A standard remotely triggered camera can be secured at a site in several ways: (1) a bungee cord is attached to the back of the camera and wrapped around a wooden stake that has been hammered into the ground (low-theft areas); (2) a locking sleeve (which makes removal of the camera more difficult) is placed over the camera, and a cable lock is run through the holes in the camera sleeve and then through a telspar post (lightweight, square-box, galvanized-steel tubing with holes along its length on all sides to facilitate mounting hardware without drilling or welding) in the ground (medium-theft areas); (3) the camera is placed inside a lockbox (making the camera more difficult to take or destroy) that has been attached to a telspar post hammered into the ground. In all instances, cameras should be situated ca. 1 m from the side of the trail and ca. 1 m off the ground (approximately mid-thigh). With the camera turned off, the height at which the motion sensor beam is broken can be tested. If the "range of tripping" of the camera beam is too high (camera too close to the trail), smaller species traveling close to the camera will typically pass by undetected. To eliminate this problem, an investigator places his or her hand on the ground at the closest point at which an animal is likely to pass in front of the camera (e.g., at the edge of the trail) and then moves his or her hand upward to the height (a few inches off the ground) at which a small animal should trip the motion sensor beam; the camera is moved away from the trail until it does so (verified when the red light on the face of the camera unit is set off) and then is secured. It is important to make sure that the beam reaches the opposite side of the path (especially on wider trails and roads), to ensure capture of animals traveling on that side of the trail. This can be done by walking along the farthest edge of the trail and seeing if the sensor is tripped.

When film is changed or retrieved from a remote camera (or when photos are downloaded or the storage disk changed in the case of a digital camera), investigators should ensure that all camera and motion sensor batteries (AA, lithium, etc.) are charged and that month, day, year, and time settings are correct. When checking the stations, the data can be entered onto a PDA form (see "Handheld Computers for Digital Data Collection," in Chapter 4). For each visit by a species, the investigator records the following information: site name; camera name/number; whether or not the batteries were replaced, the date/time stamp checked, and the film was used up (or the disk was full); and the number of photos that were taken. If film is being used, it should be changed if 75 percent or more of the frames (18 for 24- or 30 for 36-exposure rolls) have been exposed. Each roll of film should be marked with the number of the camera from which it was taken using a Sharpie or other permanent-ink pen.

Images or saved files are added to an Access database or other database system along with the following information: PhotoID (file name of the saved digital image, or write the PhotoID on the back of each photo); site (code for the site where photo was taken); date (mm/dd/yyyy) and time the photo was taken; species (species code); direction the animal is traveling in front of the camera (N, S, E, or W only); individual identity (if the animal is marked).

VIDEO CAMERAS

Video and remote-camera technology has emerged as a powerful tool for biological investigation and research, particularly when direct observation is difficult, disturbing to the animal, or a physical impossibility. Most studies using video cameras have employed fixed, mounted cameras focused on nests of birds, on burrows, or on animal movement along activity corridors, where they can monitor predators, occupancy, behavior, and predation. Several studies with video monitoring have documented snake predation at bird nests, and video cameras are becoming standard monitoring tools (Thompson et al. 1999; Morrison and Bolger 2002; Peterson et al. 2004).

Video cameras are also used as an active sampling technique at specific locations to answer particular biological questions such as the rate of occupancy of burrows of a specific shape or to identify the numbers and types of symbionts occupying a target-species' burrow. Data can be represented as percentage occupancy or occurrence in the sample. Markwell (1997) used a video camera system to observe burrow occupancy and estimate population densities of burrow-dwelling birds and tuatara (*Sphenodon punctatus*) in a variety of habitats on Takapourewa, New Zealand. Burrow cameras have also been used in animal damage control studies (VerCauteren et al. 2002) and to monitor Gopher Tortoise burrows (Smith et al. 2001).

Sandpiper Technologies markets wildlife research equipment, including the Peep-A-Roo and the Peeper Video Probe Systems (geared toward Leopard Lizards [*Gambelia*] and snakes, and Desert Tortoises, respectively, among other species). The Peep-A-Roo Video Probe is 2.5 cm in diameter and 4 m long and appropriate for the smaller burrows of snakes and lizards. VerCauteren et al. (2002) used it and discovered Western Diamond-backed Rattlesnakes (*Crotalus atrox*), Gopher Snakes (*Pituophis catenifer*), and Side-blotched Lizards (*Uta* sp.) in the dens of California Ground Squirrels (*Spermophilus beecheyi*). Milne and Bull (2002) used an Olympus optic fiber scope and light source to check the holes of burrowing spiders for the presence of endangered Pygmy Blue-tongued Skinks (*Tiliqua adelaidensis*).

Pole-mounted cameras can be used to observe animal activities in elevated, hard-to-reach locations. Richardson et al. (1999) used a TreeTop Peeper II to monitor tree cavities for Red-cockaded Woodpeckers (*Picoides borealis*). In addition to birds and squirrels, the cameras detected both rat snakes (*Pantherophis* sp.) and Red Cornsnakes (*Pantherophis guttatus*).

Camera stations reduce cost and effort, as only one person is needed to maintain them. The collection of photos or video footage minimizes field time and allows for a more detailed review of the data in the office. Cameras facilitate collection of baseline data and are efficient for monitoring and censusing in inhospitable terrain. This method allows multiple research questions to be explored at the same time and facilitates the study of arboreal and fossorial behaviors. Video cameras are reliable for nest monitoring; they are useful for identifying predators and accurately determining nest fates and total numbers of visits, and for comparing diurnal and nocturnal behaviors. The burrow cameras complement above-ground visual searches and allow one to explore dens and burrows without destroying them.

Some drawbacks of camera stations include malfunction of equipment, disturbance of light sources (regular or infrared), gnawing of cables by animals, abandonment of nests or burrows, and intense and costly labor. In addition, burrow probes are difficult to direct through 90-degree turns.

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Nest and Track Surveys

Jack Frazier

Field biologists must often resort to indirect means of sampling their study subjects or study areas. This routinely involves the observation and enumeration of *spoor,* or the signs left behind by an animal. Field studies of mammals are well known to include observations of tracks, scat (fecal droppings), or other sign; and there are many accounts, romantic and otherwise, about uncommonly skilled people "reading sign" and piecing together stories of an animal's life history (e.g., Murie 1954). Some renowned field biologists have even integrated artistic considerations into animal tracking (e.g., Ennion and Tinbergen 1967). Less well known in the popular literature are studies of reptile tracks, which are, nevertheless, well accepted and important for various species.

Target Groups

Indirect observations and measures may provide invaluable, even unique, information on certain aspects of an animal's life history. This is especially true for marine and aquatic reptiles that are usually difficult to observe and capture because of the environments in which they live, as well as their large body size, strength, and speed. Crocodilians, large chelonians (e.g., marine turtles, families Cheloniidae and Dermochelyidae), and large lizards (e.g., monitor lizards [Varanidae] and iguanas [Iguanidae]) regularly leave conspicuous tracks on the substrate, especially when it is sandy or muddy. Consequently, when these relatively large animals crawl out onto land to nest or sun, they routinely produce spoor that is easily recognizable and valuable for interpreting certain aspects of life history. However, the tracks of even relatively small chelonians, lizards, and snakes can also be studied, particularly if they occur on sandy and/or muddy substrates, at "track stations" or crossing sandy roads where direction and timing of movements of study animals can be systematically monitored (Lillywhite 1982; Dodd et al. 2004). Tracks of individual study animals, even relatively small-bodied individuals and species, can be identified when subjects are treated with a special material, such as fluorescent powder, which makes the tracks easier to distinguish (e.g., Blankenship et al. 1990; Dodd 1992b; Butler and Graham 1993; Stark et al. 2005; Tuttle and Carroll 2005). Some hunter-gatherer groups, such as the Irula tribals of Tamil Nadu, India, use reptile spoor to locate their quarry, even snakes. Moreover, information about extinct species (e.g., body size, gait, posture, speed, clues about behavior and ecology) can be revealed by paleontological tracks (Manning 2004). In the main, however, reptile tracks

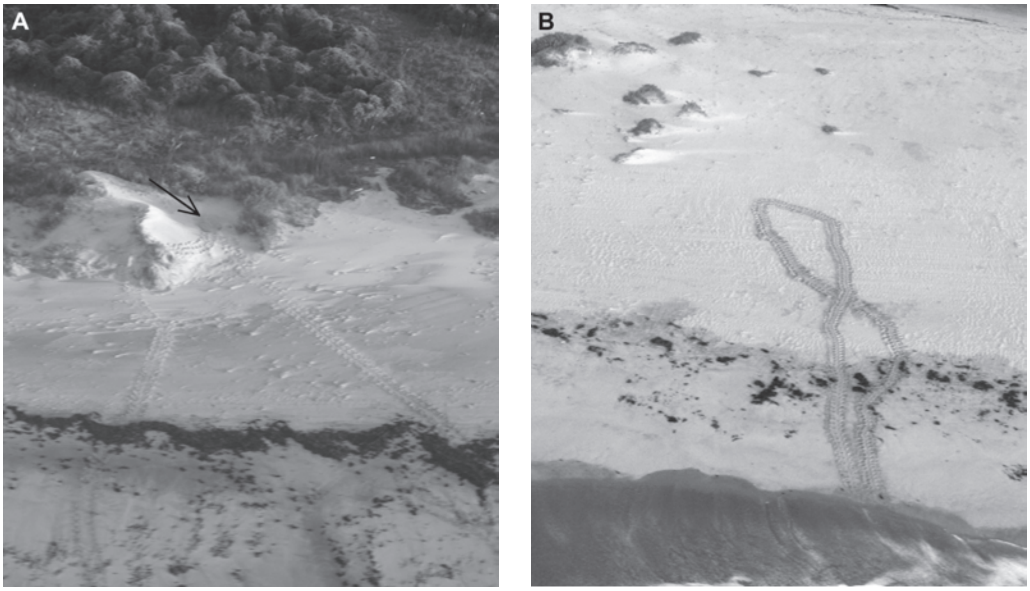

FIGURE 91 Emergences of Loggerhead Sea Turtles (*Caretta caretta*), in South Carolina. (A) Successful nesting emergence on North Island (33° 18' N, 79° 10' W; June 2007). Note the body pit at the apex of the tracks (arrow) and the returning track (on the left), between the nest site and the high-tide mark. (B) Nonnesting emergence, or "false crawl," on Lighthouse Island, Cape Romain National Wildlife Refuge (33° 00' N, 79° 23' W; June 2007). Notice the absence of a body pit and the continuous incoming to returning track, which crosses over the incoming one. (Photos courtesy of Thomas Murphy; taken from a fixed-wing aircraft about 200 ft asl.)

are most commonly used by researchers in studies of the nesting activities of living marine turtles (Fig. 91) and terrestrial activities of crocodilians.

The geographic locations of tracks provide invaluable information on the localities of major nesting, foraging, and sunning areas, and the chronology of fresh tracks provides information on the seasonality and diurnal timing of such activities. More detailed data, such as the environmental characteristics of areas where tracks are found, are invaluable for revealing the types of habitats used for various activities. Careful examination of tracks and nests can yield important data on reproductive timing, effort, and success, with the possibility of deciphering additional information on mortality and activity cycles of the study animals, as well as information on predators and predation. Estimates of animal size classes and abundance can also be derived from studies of tracks and spoor. Feces, as well as the remains of prey items, can provide information on food items, feeding areas, and habits; in recent years, feces have been used for hormonal studies and DNA-based species identifications. At the very least, correctly identified tracks and other spoor can be used to confirm the presence of certain species in a particular area.

In theory, the only equipment required for studying tracks is a notebook and a pencil. Depending on the objectives of the study, other equipment may include cord or flagging and a tape measure for quantifying aspects of the tracks or spoor and delimiting study units; a camera and a GPS unit may also be used. Most importantly, observers need a curious and inquiring mind. If large areas (scores or even thousands of km^2) are to be sampled, investigators will need transportation, such as draft animals, all-terrain vehicles, small boats, or small aircraft, to be able to cover entire areas or move between them.

Critical to the evaluation of spoor is its correct identification to species. For example, some freshwater shores host more than one species of crocodilian, more than one species of large lizard, and several species of chelonian, not to mention aquatic mammals. An investigator must be able to distinguish one track from another under a variety of conditions. Likewise, many marine beaches are visited by more than one species of nesting turtle, and in some cases sea lions (Otariidae) may haul out on the same beaches, leaving potentially confusing tracks. Making accurate species determinations of tracks and other spoor in these situations can present serious challenges, and the observer must be able to interpret subtle characteristics of the spoor. Depending on the animal, its size, posture, and behavior, as well as the type of substrate, tracks may include imprints of just the feet or of the feet together with other parts of the body, such as the tail, head, and jaws. These details help not only in identifying species but also in determining animal size class, behavior, and aspects of natural history. Muggers (*Crocodylus palustris*), for example, are capable of using the "high walk" in which the body is raised completely off the ground, whereas gharials (*Gavialis gangeticus*) cannot, so the tracks of the latter always show drag marks of the belly, whereas mugger tracks may show only footprints and tail drags (Singh 2000). Tracks of marine turtles can be identified on the basis of track width, depth, and form (e.g., asymmetrical or symmetrical), and beach type; however, different substrates and/or small differences in beach profile or angle of incline can cause a single individual to leave sets of tracks that appear entirely different (Pritchard and Mortimer 1999; Schroeder and Murphy 1999).

Other basic questions, depending on the objectives of the study, include making accurate estimates of the date and conditions under which tracks or other spoor were left and distinguishing multiple tracks of the same individual, which could be identifiable because of certain injuries or other distinguishing features. If information on size class is needed, the investigator must develop reliable methods for estimating body size from some aspect of track dimension. This means that if this information is not already available for the species, substrate, and site involved, reference measurements and observations need to be obtained from animals and tracks of known size (either in the wild or in captivity) to obtain raw data required for deriving equations for predicting body size from track size.

Background

Concise descriptions of tracks left by nesting marine turtles are given by Pritchard and Mortimer (1999); Schroeder and Murphy (1999) provide valuable explanations for developing beach monitoring programs. Additional information on identification and surveys of tracks of species that nest in Florida can be found in *Marine Turtle Conservation Guidelines,* published by the Florida Fish and Wildlife Conservation Commission (2007). Where mass-nesting beaches are involved and track densities are exceptionally high, the usual methodologies of spoor analysis may be uninformative or even deceiving (Valverde and Gates 1999). Singh (2000) described the use of crocodilian sign for distinguishing species, studying feeding and other activities, and determining body size. The amount of detailed information on field techniques using tracks is greatest for marine turtles, so that information will form the bulk of the following synopsis. Nevertheless, the general principles of research design, field methods, resources, and data treatment are comparable for other, small-bodied reptile species (e.g. Lillywhite 1982; Blakenship et al. 1990; Dodd 1992b; Butler and Graham 1993; Doddy et al. 2003; Dodd et al. 2004; Stark et al. 2005; Tuttle and Carroll 2005).

Research Design

As Schroeder and Murphy (1999) explained, although beach surveys—primarily the enumeration of tracks left by nesting females—are widely employed in marine turtle studies, the value of the data gathered is greatly reduced by the variability of methods used, lack of documentation of techniques, and inappropriate assumptions that are often made. They noted (p. 45) that "[i]n order to be of value over a long period of time, surveys on nesting beaches must be cost-effective, reproducible, quantitatively rigorous, and easily taught to others who will continue the surveys."

Critical factors that must be considered in any beach survey include the following: species-specific differences in spoor, nesting behavior, and habitat use; nest density; beach type; time of day (tide and shadows); wind; rainfall; tides (lunar phase); beach disturbances (activities by animals and humans); and observer accuracy. Some turtle species (particularly Hawksbill Sea Turtles [*Eretmochelys imbricata*]) will crawl over hard substrates (e.g., beach rock or coral rubble) or nest under dense vegetation, leaving tracks that are difficult to detect or interpret. Other species (particularly Ridley Sea Turtles [*Lepidochelys* spp.]) leave very light tracks that are visible for relatively short periods; and some species (e.g., Green Sea Turtle [*Chelonia mydas*] and Leatherback Sea Turtle [*Dermochelys coriacea*]) dig up vast areas of beach, covering over and destroying their own spoor and that of others. If nesting activity is intense, beach spoor may be too confused to interpret; this is especially problematic with Ridley and Green Sea turtles on certain beaches. Hard-packed and/or coarse sand are poor substrates for tracking. Spoor, particularly tracks, can be accentuated by shadows that provide contrast and greatly increase visibility; on the other hand, glare from reflection off the sea or beach can hamper visibility. These considerations are especially critical in aerial surveys, where tracks can simply appear and disappear depending on the angle of observation and shadow position (see "Aerial Surveys for Marine Turtles" in this chapter, below). Tides can wash away large proportions of tracks or, on the other hand, expose vast expanses of intralittoral substrate, greatly altering what is visible and what is available for inspection. Hence, time of day as well as tidal cycle can have major effects on track visibility and survey results. In general, it is best for investigators to traverse a beach early in the morning when the sun is low and contrast from shadows is highest. The integrity of tracks, and hence their visibility, is damaged by wind and rain, both of which erode spoor, although tracks made after a light rain can be singularly conspicuous. Thus, the results of surveys must be interpreted in the light of weather conditions. A wide variety of animals cross beaches and use them for roosting, burrowing, or foraging, and these activities can confuse or obscure tracks and other spoor; likewise, human activities, particularly humans walking or driving across an area, can obliterate tracks. On top of all these factors, differences in methods used as well as observer experience and competence can result in significant differences in track interpretation.

Observer variability and bias is likely to be one of the most important sources of error and variation. Unfortunately, training of observers for beach surveys is often ignored or given inadequate attention despite being essential to ensure the use of standardized methodology as well as to reduce bias and errors. Observer training is fundamental for increasing comparability between studies and between areas and times within the same study. To be most useful, results should allow for the interpretation of trends, and this requires comparisons over years, if not decades, using standardized methodology. That means that results from different observers, study areas, and sampling periods will be compared, and investigators must be able to separate biological differences from differences related to variability in the way the data were obtained and/or interpreted.

Ground truthing is the process of collecting data in the field (on the ground) at a particular site and comparing it with data collected for the same area using some type of remote sensing (e.g., aerial photography, satellite imagery) in order to determine the congruence of the data collected by the two different methods. Ground truthing is essential for standardization of data and techniques; individuals making the observations on the ground as well as those making aerial observations need to be adequately trained. It is critically important to evaluate observers' abilities to identify the species responsible for tracks, as well as to determine the date they were made or, at least, the "freshness" of the tracks. In many cases it is also necessary to evaluate observers' abilities to determine accurately whether or not a track is from a successful nesting attempt (i.e., whether or not eggs were deposited). To be useful, evaluations of observers (both ground and aerial) must be carried out repeatedly during a season of surveys and under a variety of conditions (e.g., beach type, tide, weather, etc.); the evaluations should be conducted by highly experienced personnel. Ground truthing should be used with every aerial survey, to establish the observers' abilities to identify species correctly and to distinguish successful nesting attempts on the basis of what is known to have happened on the ground on the beach being surveyed.

Survey or monitoring efforts must be organized in both time and space. In many cases, surveys cannot be conducted on a daily basis but are done much less frequently, and the results are used to extrapolate information for an entire season. The periodicity and timing of surveys must take into account the various critical factors described earlier, ideally involving as many surveys in a season as possible and spanning the period with the most animal activity. Beach surveys for marine turtle nesting should start in the early morning to take advantage of fresh tracks, low angle of the sun and resulting high light contrast, and shortest exposure to wind, tides, and other events or activities that can obliterate tracks. Surveys conducted the day after a high tide that has peaked just after dark can take advantage of having a "clean slate" with conspicuous fresh tracks below the high tide line.

Certain crocodiles and lizards tend to forage and/or bask along shorelines, so if fresh sign is needed, track surveys for these animals can be conducted after those periods of greatest activity. Nonetheless, morning surveys still provide advantages over afternoon surveys, including contrasting light and shadow and reduced daytime disturbance of spoor.

Coastlines, because they extend for hundreds of kilometers, are more manageable if organized or partitioned into sectors for sampling. Certain sectors can then be used as "index beaches" and subjected to regular, long-term monitoring to evaluate trends over time. These special sectors must be accurately located and carefully delineated so that the amount of area being sampled is accurately known. Information derived from areas of known size allows density values to be estimated reliably. The limits of each sector must be constant over the years, which means that boundary markers must be accurate and permanent. GPS technology is especially useful in that regard. Beach-survey sectors should not generally exceed 1.0 km in length (Schroeder and Murphy 1999). In any case, it is essential to bear in mind that beaches are highly dynamic, and various characteristics can change from one season to another or even within a season.

Field Methods

The use of data forms not only increases the efficiency with which data are recorded but also ensures that all essential parameters are logged and that coding and observations are standardized (for examples, see Schroeder and Murphy 1999). Standardized data forms can be created on paper or on handheld electronic devices (see "Handheld Computers for Digital Data Collection," in Chapter 4), and stored for permanent reference.

Beach surveys conducted on the ground allow more detailed scrutiny of tracks, nests, and other spoor than do aerial surveys; they also accommodate other activities such as predator monitoring and control. Where vegetation or substrates obscure the spoor or small aircraft cannot land safely on the beach, separate ground surveys may be required. On the other hand, aerial surveys allow investigators to inspect scores of kilometers per hour and thereby cover relatively large areas in short periods; they also allow for observations of relatively isolated and inaccessible areas.

Small fixed-wing aircraft and helicopters can be used to survey crocodile tracks and nest mounds, if accessibility and visibility are adequate. In addition, investigators often survey crocodilian spoor from small, shallow-draft boats that can navigate close in and parallel to the shoreline. Such craft can be directed to nests and other data-collection sites from a helicopter. Whatever the procedure, ground surveys are required for ground truthing, if not for the major part of the survey. An index of population size for breeding females is often derived from nest-mound surveys, so it is essential that survey methods be standardized.

Nesting-beach surveys, for a variety of animals, can be used to enumerate all nest spoor or only "fresh" spoor produced during the previous 24 hours. Researchers can also differentiate between tracks that lead to or from nests with eggs (clutches), that is, between "successful nestings" on the one hand, and unsuccessful nesting emergences on the other. For marine turtles the latter are called "*false crawls,*" "*half moons,*" or "*spaghetti tracks*" (Fig. 91B). If spoor that has accummulated over days or weeks is counted during a single reconnaissance, then distinction between successful and unsuccessful nestings should not be attempted, for nesting success cannot be accurately determined. In all cases, it is essential to ground truth the data to verify the quality of the observations and to define the relationship between the interpretation of spoor and what has actually happened on the ground.

Several methods can be used to distinguish between fresh and old tracks and to avoid duplicate counts. The day before a survey is to be conducted, a beach sector can be marked with a line (by foot or vehicle) just above, and parallel to, the high-tide mark; the observer can then distinguish fresh tracks (those that go over the line) from old spoor (those that the line crosses over). To avoid double counting, spoor that has been enumerated can be "scratched off" by putting (or leaving) an obvious mark on top of the track.

The route and speed of an observer surveying a beach must be carefully planned. Just above the high-tide line, both the emerging and returning tracks should be readily visible; below the line, tracks can be washed out by tides and high seas, depending on tidal cycles and weather conditions. Higher up the beach, vegetation, dunes, and blowing sand may interfere with the interpretation of spoor. Mechanized travel (ground, aquatic, or air) must not exceed observers' abilities to detect, differentiate, and record sign. Flying speed generally should not exceed 100 km/h (about 60 knots, or 69 mph). Flight altitude can range from about 60 to 250 m, depending on relative ground speed, beach characteristics, and the type and density of spoor under observation.

Aerial surveys are most effective if conducted with low, direct sunlight and in conjunction with the best tidal phase for the beach involved. Boat and all-terrain-vehicle (ATV) surveys need to consider tides and light direction to ensure both accessibility and visibility.

Resources Required

As mentioned above, the training of observers is essential for standardizing the way data are collected and interpreted. In this respect, pilots of aircraft and boats and drivers of terrestrial vehicles should be informed about the objectives and methodologies of the work, because the trajectory of the observers' platform is intimately related to the quality of the observations. Repeatedly employing the same pilot who is experienced in the survey methods and routes is preferable. All participants in field surveys, whether observers or pilots, must avoid working while fatigued. Too often, fieldworkers have a "Rambo ethic" in which super-human physical virtues are idolized and mortal aspects of fatigue, mistaken decisions, and error are ignored, if not denied. Observer concentration and sensitivity diminish with fatigue, and data gathered by fatigued observers, no matter how much they espouse the Rambo ethic, will be unreliable. In general, observers, pilots, and others involved in a survey should stay focused on the task, refraining from idle chatter and unnecessary attention to objects unrelated to the survey.

While training manuals (such as this one) and training sessions are invaluable, the most effective form of training is through an apprenticeship with experienced, reliable investigators. Experienced but unreliable people should not be used for training!

Data Treatment And Interpretation

Surveys provide population estimates for evaluations of a species' status in the short term; using survey data to interpret medium- and long-term population trends is more challenging. Because of the number of variables that can affect the activities of a population and the longevity/survivorship of the individuals—not to mention the variability in "nonbiological" factors—multiple surveys of an area using standardized techniques must be carried out over years, if not decades, to provide a basis for determining population trends. It is important to back up data regularly and store them in (multiple) secure locations.

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Aerial Surveys for Marine Turtles

Robert D. Kenney and C. Robert Shoop[1]

We define *aerial survey* as visual assessment of a population of animals or their sign by one or more observers in an aircraft; we do not include photography, radio tracking, or other nonvisual applications of aerial platforms. The aircraft can be a fixed-wing airplane, a helicopter, or a lighter-than-air airship (more commonly known as a "blimp"). It can be as small as a one-person "ultralight" airplane, but that would obviously limit the area that could be covered.

Aerial surveys are the commonly preferred method for assessing populations of animals that inhabit large areas, because they enable investigators to cover long distances or large areas in a relatively short time. This capacity makes aerial surveys particularly useful for assessing sea turtles, whose nesting beaches span tens to hundreds of kilometers of coastline and whose populations range over thousands of square kilometers of oceanic habitat (Shoop et al. 1985; Shoop and Kenney 1992; Meylan 1995; Henwood and Epperly 1999). Aerial surveys for sea turtles can be conducted with a variety of different objectives. We will discuss two principal categories of aerial turtle surveys—nesting beach surveys, whose principal objective is assessing the occurrence and intensity of nesting activity, and over-water surveys, flown with the objective of assessing the occurrence, distribution, and abundance of sea turtles in their oceanic habitats.

Definitions

NESTING BEACH SURVEYS

Observers conducting aerial surveys of nesting beaches fly the length of a beach, searching for visible evidence of sea turtle nesting activity—that is, primarily tracks ("crawls"), but also the nesting sites themselves. For measures of relative nesting activity, aerial surveys can provide indices of nesting intensity (from as simple as presence/absence to numbers of nests per km of beach and specified time period) with minimum human effort, especially for remote beaches or over extensive distances (Shoop et al. 1985). The distinctive fan-like array of tracks left by hatchlings emerging from a nest can also be observed under the proper conditions (good visibility; flat, undisturbed, sandy beaches; slow aircraft speed; low altitude; and optimal lighting conditions) and used to develop an index of hatching success.

OVER-WATER SURVEYS

Aerial surveys of populations of in-water sea turtles can vary greatly in scope and complexity. They may be simple, such as an attempt to detect the presence/absence of turtles in a defined, local area at a specific time, for example, prior to some specific activity (e.g., a military exercise, dredging, explosive removal of oil rigs, fishery opening/closing). If turtles are present, steps can be taken to avoid subjecting them to possible adverse impacts. At the other extreme, aerial sea-turtle surveys can provide detailed assessments of the spatiotemporal distributions and abundances of multiple species over wide geographic areas and multiple seasons or years.

Target Groups

NESTING BEACH SURVEYS

Because observers on aerial beach surveys are searching for tracks rather than directly for turtles, they must be able to identify species from crawl patterns or patterns of nesting disturbance. More significantly, they must be able to determine whether an observed crawl truly represents a nest. Pritchard and Mortimer (1999) described details of crawl characteristics for various species of marine turtles. Generally, Leatherback Sea Turtles (*Dermochelys coriacea*) and Green Sea Turtles (*Chelonia mydas*) move both forelimbs simultaneously when crawling, as opposed to other species, which alternate the use of their front limbs ("butterfly" vs. "freestyle"). Green Sea Turtles generally dig deeper body pits than Loggerhead Sea Turtles (*Caretta caretta*) or ridleys (Olive Ridley Sea Turtle [*Lepidochelys olivacea*], Kemp's Ridley Sea Turtle [*L. kempii*]), causing more disturbance. Leatherback tracks are significantly wider and deeper than those of any of the "shelled" turtles. Hawksbill Sea Turtles (*Eretmochelys imbricata*) often nest on hard, coral beaches in vegetation, which decreases track visibility. Tracks of emerging hatchlings of all species are too small to be identified to species from the air.

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[1] deceased

OVER-WATER SURVEYS

Detectability of a sea turtle during an over-water aerial survey is principally a function of its size. In an aerial survey experiment conducted in 1984 (Schroeder and Thompson 1987; N. B. Thompson, pers. comm. in Shoop and Kenney 1992), investigators showed that turtles with carapace lengths of less than 60 to 75 cm were difficult to detect from a fixed-wing aircraft flying at an altitude of 500 ft (150 m) and speed of 100 to 120 knots (185–220 km/h). Larger animals are seen more easily; therefore, surveys are most effective for turtles the sizes of large juvenile to adult Loggerheads, Greens, and Leatherbacks. Decreasing altitude increases the likelihood of spotting smaller targets. However, it also reduces the field of view and causes the apparent speed to increase, which means that less of the ocean surface is in view and that it is visible for a shorter period. Flying more slowly reduces the effects of decreased altitude, but there are limits to how slow fixed-wing aircraft can safely fly. RDK (unpubl. data) conducted an experiment to assess dolphin and sea turtle sightability off the east coast of Florida on 15 January 2001; he used an airship flying at 1,000 ft (305 m), to duplicate the altitude used for whale surveys, and about 35 knots (65 km/h) ground speed. Given optimal sea state (calm seas with no whitecaps) and sun glare (high sun angles during midday and on the opposite side of the aircraft) conditions, and using high-quality 10X binoculars, he and his crew detected and positively identified Kemp's Ridley Sea Turtles with carapace lengths estimated at 25 to 30 cm.

Because species identification can be difficult, observer training and experience are critical to project success (see Henwood and Epperly 1999). It is generally easiest to differentiate leatherbacks from the shelled sea turtles, based on size, color, and body shape (Pritchard and Mortimer 1999; Wynne and Schwartz 1999). Loggerheads are relatively distinctly colored, as are Kemp's Ridleys (if they are large enough to be seen in the first place). Depending on the number of species that occur in a given area, it may frequently be necessary to classify sightings as "unidentified cheloniid sea turtles." Marsh and Saalfeld (1989) found, for example, that during an aerial survey from an altitude of 450 ft (140 m) over part of the Great Barrier Reef in Australia, observers could not reliably differentiate among the five species of cheloniid sea turtles (Loggerhead, Green, Flatback [*Natator depressa*], Olive Ridley, and Hawksbill) known to be present.

Background

NESTING BEACH SURVEYS

Aerial beach surveys have commonly been used to assess the size or status of sea turtle populations. Meylan (1995) described a standard method for using a combination of aerial and ground counts of nests as an annual index of the number of nesting females; extrapolating from that, she estimated the total number of adult females and total population size (see also Schroeder and Murphy 1999). Pritchard (1982), in contrast, after a 2-day survey for Leatherback nesting crawls along approximately 1,000 km of beach on the Pacific coast of Mexico, concluded that it was not possible to extrapolate accurately to the number of nesting females in the population.

Shoop et al. (1985) reported counts of nesting crawls from Cape Hatteras, North Carolina to the U.S./Mexico border in May, June, and July 1982, perhaps the largest nesting beach survey ever conducted. Smaller-scale aerial nesting surveys are more common; early projects were reported by Carr and Carr (1977), Davis and Whiting (1977), and LeBuff and Hagan (1978). Crouse (1984) described 2 years of summer surveys of North Carolina beaches. Reports of small-scale, short-term aerial beach surveys for turtle species are often included in the proceedings of the annual symposia on sea turtles (e.g., Hopkins-Murphy and Murphy 1988, 1994; Mayor et al. 1998; Ross 1998; Sarti et al. 1998, 2000; Limpus et al. 2002; also available online at http://www.nmfs.noaa.gov/pr/species/turtles/symposia.htm). Aerial surveys of beaches can also be used to locate stranded, injured turtles or turtle carcasses for assessment of the impacts of fisheries on sea turtle populations (Booker and Ehrhart 1989, 1990).

OVER-WATER SURVEYS

Monitoring sea turtle populations with small-scale surveys is common, but the survey results are rarely published beyond the gray literature of internal government agency reports. Klima et al. (1988) reported briefly on the use of helicopters to survey sea turtles and marine mammals in the areas around oil-production platforms in the Gulf of Mexico prior to their explosive removals. Surveys covering an area within a 1.5-km radius around the platform were carried out within 1 h prior to a detonation; if turtles or mammals were sighted within 915 m (1,000 yd) of the platform, the detonation was delayed by 1 h. Dodd and Mackinnon (2002) described aerial surveys regularly flown in April and May in Georgia coastal waters in an effort to document and mitigate excessive fishery-related mortality of Leatherback Sea Turtles. Sighting rates greater than 10 turtles per 50 km of trackline triggered a review to determine whether fisheries should be shut down or other restrictive measures instigated.

One of the first major, large-scale aerial-survey programs was the BLM (U.S. Bureau of Land Management) Cetacean and Turtle Assessment Program, or CETAP (few large-scale aerial surveys have been dedicated exclusively to sea turtles). Under CETAP, University of Rhode Island biologists carried out year-round aerial surveys of the continental shelf waters off the northeastern United States (approximately 280,000 km^2) in 1979 through 1981. They used line-transect survey methods to assess the distribution, density, and abundance of cetaceans and sea turtles in areas of potential oil and gas development (CETAP 1982; Scott and Gilbert 1982; Shoop and Kenney 1992). Fritts et al. (1983) reported on a similar multispecies aerial survey project carried out in 1980 and 1981 off the coast of the southeastern United States. Their surveys were not broad scale and continuous over the region but focused instead on four separate survey blocks (approximately 25,000 km^2 each)—off eastern Florida, southwestern Florida, Louisiana, and Texas.

In the United States, the National Marine Fisheries Service (NMFS) in the Department of Commerce has primary responsibility for management of sea turtle populations. NMFS conducted large-scale surveys from 1982 to 1984, specifically targeting sea turtles from southern Florida to North Carolina (Schroeder and Thompson 1987). Other dedicated sea turtle surveys conducted by NMFS have covered much smaller areas; for example, the NMFS lab at Beaufort surveyed the coastal and estuarine waters of North Carolina as part of a sea turtle research program of much broader scope (Braun et al. 1990; Epperly et al. 1991; Epperly, Braun, and Chester 1995; Epperly, Braun, Chester et al. 1995; Braun and Epperly 1995). Frequently, sea-turtle-sighting data collected during NMFS marine-mammal-specific surveys have been used afterward for sea turtle assessments (McDaniel et al. 2000; Mullin 2000; Palka 2000). Lohoefener et al. (1988) and Fuller and Lohoefener (1990) reported data on sea turtles sighted in the water, stranded, or on nesting crawls during aerial surveys designed primarily for fishery purposes.

There have been various other small-scale over-water sea-turtle aerial surveys. Researchers from the Virginia Institute of Marine Sciences have flown turtle surveys both in Chesapeake Bay and off Cape Hatteras (Keinath et al. 1987; Coles et al. 1994; Musick et al. 1994). Carson (2000) reported on surveys of the near-shore waters along Palm Beach County, Florida. Grant et al. (1996) flew near-shore surveys along Topsail Island, North Carolina. Several aerial survey projects have been conducted in Australian waters—off the Northern Territory (Bayliss 1986), around the Great Barrier Reef (Marsh and Saalfeld 1989), and in a small experimental area (Marsh and Sinclair 1989). The primary target of the Australian surveys was the Dugong (*Dugong dugon*), with sea turtles as a secondary objective.

Research Design

NESTING BEACH SURVEYS

Aerial beach surveys, by covering long distances within a few hours, result in indices of nesting activity with minimal bias from variability in daily environmental conditions. Regardless, a large number of other variables can influence the accuracy of the data obtained. A discussion of aerial nesting surveys is actually a treatise on minimizing the effects of those variables. Most aerial nesting surveys are not carried out daily; hence, they are "snapshots" of activity that do not encompass the unpredictable, daily variability.

Marine turtle tracks leading to and from putative nests can be counted during aerial surveys, but observers must be able to recognize the *nest crawls* (tracks of nesting turtles) and sometimes their different species characteristics as an aircraft maneuvers along a nesting area. The main question facing an observer is whether the crawl represents a nest, a decision that must be made with only a few seconds of observation. Sea turtles sometimes fail to nest, producing false nesting tracks, or *false crawls* (see Fig. 91). Sometimes those false crawls are obvious as "U" turns on the beach, but other crawls are misidentified even by trained and knowledgeable observers on the ground. Observers often separate *fresh crawls* made the night before a survey from tracks made previously (*old crawls*) to obtain a count of 1 day's nesting activity. If such differentiations are needed, criteria that will minimize inclusion of false and old crawls must be established. Frequently, flying surveys shortly after dawn the morning after the new- or full-moon tides (higher spring tides), which obliterate the visible old crawls and U-turns, reduces the possible confusion and/or error. We caution, however, that repeated daily surveys of the same beaches have shown that old tracks in hard sand that have been filled and obliterated by blowing sand can reappear days later and appear "fresh" from the air.

Often, aerial surveys are enhanced by *ground-truth teams* that check the accuracy of aerial observations and provide "correction factors" for use in final data analyses. Such corrections may be of limited value because so many other variables are involved. Schroeder and Murphy (1999), nevertheless, considered ground truthing critical for data analysis and provided details on carrying out such surveys. Obviously, if ground-truthed data are available for the entire area of concern, then no aerial surveys are needed. However, comparisons of data from aerial surveys with those taken on the ground are useful for assessing the accuracy with which aerial observers can differentiate nesting crawls from false crawls, developing correction factors applicable to other areas and times, and so forth. Observers should follow a set of criteria for distinguishing crawl types in order to standardize the data taken, while acknowledging that some errors will be made.

We have found that more than 90 percent of the crawls that lead into vegetation, include thrown sand, or end in areas of substantial disturbance are associated with a nest. The separate tracks up the beach and back down again of new nesting crawls usually are of unequal length, particularly if the nest was made under a new or full moon, when tidal ranges are at their maxima. The occurrence of rain or strong winds following egg deposition, a rocky or pebble beach that precludes flipper impressions, beach cleaning by humans, and feral-animal tracks can make the species identification and/or classification of crawls difficult.

Periodic aerial surveys, even if accurate, cannot account for daily variability in nesting and hatching numbers. Unless areas are surveyed daily, the data obtained are indices of nesting or hatching activity rather than precise counts of nests or hatchlings at given areas or times. They can be used to determine relative nesting or hatching activity between areas or times. In some cases, simple crawl counts (including false and old tracks) are sufficient to distinguish areas of heavy nesting activity from those where it is low.

OVER-WATER SURVEYS

Research design is rarely a significant issue with small-scale surveys designed for impact monitoring or mitigation projects. Typically, the expected area of influence or impact is predefined based on the type of activity involved. The "design" is to saturate the area with survey coverage to the extent practical. In many cases, it will not even be necessary to identify species; rather, the principal objective is simply to detect the presence of any turtles.

In contrast, aerial surveys intended to estimate population abundance are complex and expensive. They should not to be undertaken without ample resources and a great deal of advance planning. Gerrodette and Taylor (1999) and Gerrodette (2000) have provided good introductions to transect sampling methods as they apply to sea turtles. Buckland et al. (1993) discussed the same issues and mathematical treatments for the data in much more detail (see also Gates 1979; Seber 1982; Burnham et al. 1985; Laake et al. 1993; Garner et al. 1999; and "Transect Surveys, Including Line Distance," above, in this chapter). We recommend that investigators planning surveys to estimate abundance visit the St. Andrews University (Scotland) website (http://www.ruwpa.st-and.ac.uk/distance/), where one can download the full text of Buckland et al. (1993) and other reference information, a complete analytical software package (program DISTANCE), and software support and documentation, as well as find information on a variety of training courses.

TRANSECT SAMPLING The objective of transect surveys is to estimate the density (number of individuals per unit area) of animals in a population within a specified region, either the known range of the population or some arbitrarily defined study area. Density (*d*) is estimated from the number of animals (*n*) within a sample of known area (*a*), of the range or habitat:

$$d = n / a \tag{1}$$

With multiple samples, the average density (*D*) for all *S* samples is

$$D = (d) / S \tag{2}$$

If the sample areas are not all of equal size, then the mean density calculation should be weighted by sample area. The population abundance (*N*) within the entire range or defined region of known area (*A*) is then

$$N = D \times A \tag{3}$$

Density estimates from aerial surveys can be derived using two different methodologies. In *strip-transect sampling,* observers count all animals within a strip of defined width (*W*) on each side of the flight track. The area sampled (*a*) is $2 \times L \times W$, where *L* is the length of the transect, and the estimated density is then

$$d = n / (2 \times W \times L) \tag{4}$$

For social species, which normally occur in groups, the number of groups times mean group size is generally used rather than number of individuals. Although in many cases the strip width is predefined with little or no quantitative basis, the right-angle distance from the transect to each animal observed should be measured accurately in order to determine the appropriate strip width more precisely. In many cases, downward visibility for observers watching out the side windows of the aircraft is restricted, so the strip that is sampled is actually offset from the transect line. For example, McDaniel et al. (2000) surveyed a 200-m-wide strip with lateral boundaries located 50 m and 250 m from the trackline, respectively. The strip-transect method assumes a 100 percent probability of detection of all animals within the defined strip and ignores all animals sighted outside of the strip.

This assumption of 100 percent probability of detection is violated because of two types of known bias (Marsh and Sinclair 1989). During marine mammal and sea turtle surveys, some animals are submerged when the aircraft passes over and are therefore not available to be seen by an observer (*availability bias*). Other animals may be present on the surface but still be missed by an observer (*perception bias*). A primary component of perception bias is distance—in general, the farther an animal is from the transect line, the lower the probability that an observer will see it. Perception bias is also affected by the speed and altitude of the aircraft, size of the animal, number of animals in a group, experience of the observers, observer fatigue, sea state, weather, visibility, sun glare, and so forth.

The *line-transect-survey method* can be used to estimate density even if many animals are missed. As the aircraft flies along the transect line**,** the observer measures the perpendicular distance (*x*) to each sighting. A detection probability function ($g(x)$, the probability of detection at a given distance from the transect) is derived by evaluating the goodness-of-fit between the distribution of observed perpendicular distances and a variety of alternative statistical models, assuming that the detection probability on the trackline is 100 percent (or $g(0) = 1$). This $g(x)$ function in turn is rescaled into a probability density function $f(x)$ that integrates to 1 over the entire range of *x,* which is then solved for $f(0)$, or the *perpendicular distance probability density function evaluated at zero distance.* The reciprocal of $f(0)$ is *ESW*, the *effective strip width,* which is equivalent to *W* in strip-transect methodology; therefore, the line-transect method is essentially a sophisticated statistical means of measuring the area sampled. The area sampled can be expressed in two ways:

$$a = 2 \times L \times ESW \tag{5}$$

or

$$a = (2 \times L) / f(0) \tag{6}$$

Therefore, sample density can be calculated by either of two equivalent formulas:

$$d = n / (2 \times L \times ESW) \tag{7}$$

or

$$d = [n \times f(0)] / (2 \times L) \tag{8}$$

The consensus is that line-transect surveys are preferable to strip-transect surveys. Observers usually overestimate their sighting efficiency when no attempt has been made to quantify it. For example, Grant et al. (1996, p. 112) conducted small-scale aerial surveys in North Carolina coastal waters, and "turtles seen on either side of the aircraft, up to about 250 m, were counted." They concluded that "it is unlikely observers missed sighting Leatherbacks during the aerial censuses," despite not measuring sighting distances and flying at varying altitudes. Predefined strip widths for strip-transect surveys similarly tend to be overestimated, resulting in negatively biased densities. A commonly used strip width for sea turtle surveys is 200 m (Bayliss 1986; Keinath et al. 1987; Marsh and Saalfeld 1989; Marsh and Sinclair 1989; Braun et al. 1990; McDaniel et al. 2000). Barlow et al. (1988) used a predefined 182-m strip in one year, and a 412-m strip in another year during surveys for harbor porpoise (which are similar to sea turtles in sightability), without clearly explaining the reasons for the different selections. These values seem close to values of *ESW* estimated from several line-transect surveys (23–124 m, Heppell 2000; 220 m, Palka 2000; 121–338 m [calculated from other data in the report], Mullin 2000; 349 m, CETAP 1982). However, the proportion of turtles missed within a strip has been variously estimated to range from 7 percent to more than 80 percent (Bayliss 1986; Marsh and Saalfeld 1989; Marsh and Sinclair 1989), and turtles beyond the strip are ignored. Observers on line-transect surveys record turtles out to the limit of detectability in order to sample the calculated ESW effectively. Therefore, perpendicular distances should always be measured so that strip width can be defined precisely, in which case the apparent logistic advantage of strip-transect surveys disappears. It is likely that an accurate strip width will be so narrow that the level of coverage and number of sightings will be very small and the density estimates highly variable (Eberhardt et al. 1979). Line-transect methods eliminate the need for strong assumptions about detectability functions. All of the sightings are included in the data analysis (Eberhardt et al. 1979; Burnham and Anderson 1984; Barlow et al. 1988). Consequently, line-transect surveys almost always result in estimates with lower variances and greater precision (Burnham et al. 1985).

Because of the inherent assumption of 100 percent detection probability ($g(0)=1$) on the transect, the line-transect survey method does not completely eliminate bias. It does address the component of perception bias caused by distance, but not that caused by other factors, and it does not address availability bias at all. Methods for estimating the true value of $g(0)$ are available; the reciprocal of that value, $1/g(0)$, can be applied to density estimates as a correction factor. Given sufficient room in the aircraft, one can use simultaneous independent observer teams and apply an extension of mark-recapture methods to the animals sighted by one team and resighted or not by the other (Borchers et al. 1998; Borchers 1999). Alternative methods using the same mark-recapture theory include surveying with two aircraft in tandem (Hiby and Lovell 1998) or circling back after each sighting for a second pass (Hiby 1999). Depending on the time between the first and second sightings relative to typical dive durations, the latter methods may also correct for some portion of the availability bias. A different correction for availability bias (i.e., a dive correction) can be made if quantitative dive-and-surface data are available. The probability of an animal being in view is (Eberhardt et al. 1979):

$$P=(s+t)\ /\ (s+u) \tag{9}$$

where s is the time spent on the surface, u is the time spent submerged, and t is the time a given point is in the observer's field of view. If t is very small relative to s and u, P approaches the average proportion of time spent at the surface. The correction factor is the reciprocal of P (see Musick et al. 1992; Hain et al. 1999). More statistically complex and precise models can be developed (e.g., Barlow 1999).

OTHER CONSIDERATIONS Additional factors must also be considered when designing surveys. To be statistically reliable, the probabilities of sampling any point must be equal (i.e., random samples). This can be accomplished with randomly placed transects or with systematically spaced transects with a random start point. Precision of the estimated mean density or abundance is maximized (i.e., variance minimized) by orienting the transects perpendicular to any gradient in population density, which for wide-ranging marine species typically means transects perpendicular to bathymetric contours. With enough prior information about expected densities in different habitat subareas (e.g., depth zones), surveys can be stratified to increase precision further. Stratified sampling effort should be greater in strata with higher densities, generally weighted by the square root of the expected density. Transects can be parallel, which results in cross-legs between transects that are not included in the sample, or in a "sawtooth" pattern without cross-legs. Enough transect length should be sampled to yield sufficient sightings to minimize variances, minimally, 25 sightings per species, and ideally, 40 to 100 or more (Eberhardt et al. 1979). Given large enough sample sizes, separate $g(x)$ models for different combinations of sighting conditions (sea state, cloud cover, sun glare, visibility) can be derived, further improving the precision of the population estimates.

Field Methods and Resources

NESTING BEACH SURVEYS

Survey teams should include at least three people—a pilot, one person to scan the beach and make judgments of turtle crawl types, and a third person to record the data. Ideally, the recorder should have a computer with direct input of time of day and location data from a GPS (global positioning system) receiver to associate with each crawl or set of crawls. Manual data recording is possible, but it greatly increases the likelihood of errors, which can be introduced during both the initial recording of the data and when the data are converted from the paper log sheet to electronic format. It may be necessary to pass over densely packed crawls in high-activity areas repeatedly to obtain accurate counts, depending on the speed of the aircraft. Aircraft type (fixed-wing or helicopter), speed (60–100 knots), and altitude (200–800 ft [60–240 m]) can affect visibility, as can time of day (angle of sun), weather (cloud cover/sun, precipitation, haze, fog), glare, blowing sand, and other conditions in the nesting area. Optimal survey altitudes of 400 to 500 ft (120–150 m) are similar to those for over-water surveys, with descent to lower altitudes whenever necessary to get a better look at a particular set of tracks. For aerial beach surveys of hatchling-turtle tracks, the best time to fly may be shortly after sunrise, when the very low sun angle produces strong shadows in the shallow tracks. Pilots should consistently fly in the same direction (i.e., with the same side of the aircraft oriented toward the beach).

Aircraft speed and fuel capacity will determine the maximum distance that can be covered in a single flight. Funds will determine the total number of flights possible, the interval between flights, and the study duration. Some level of ground truthing should be conducted to verify the aerial survey's counts of fresh, old, and false crawls and the identifications of nesting species. Feedback from the ground-truth personnel to the aerial survey crew will help in standardizing the survey data and reducing errors.

OVER-WATER SURVEYS

The minimum crew for an aerial transect survey is four: a pilot, two observers (one on each side of the aircraft), and a data recorder. With larger aircraft and more space, additional observers can be deployed, either as independent observer teams and/or as replacement observers so as to minimize observer fatigue. We also recommend a co-pilot to share flying duty for long survey days in an aircraft not equipped with an autopilot.

Computer datalogging maximizes the frequency and accuracy of data acquisition, although fully manual data recording has been used successfully. A GPS receiver can capture data on position, time, heading, speed, and altitude. The exact setup for data recording will depend on crew size and aircraft arrangement, with several options available. An untended computer (which could be located where it is not easily accessible during a flight) might record only GPS data automatically at frequent intervals (e.g., 1 s, 5 s), with all other data recorded manually in logs. The manually logged data can be merged into the computer dataset afterward by matching times, as long as the recorder writes down times to the second whenever possible and the clocks of the recorder, computer, and/or GPS are synchronized. Another option is full computer data entry, in which the data recorder keys everything directly into the computer (which continues logging GPS data when nothing else is happening). This can become difficult during times when sightings are frequent. An off-duty observer in a larger aircraft could keep a paper log as a backup. A third option is to use a datalogger program designed to automatically record GPS data at regular intervals (30 s or 60 s would be adequate), as well as on demand, with all information at each on-demand record entered as a text string rather than trying to key in specific items in a particular order under often hectic conditions. An audio recorder connected to the aircraft intercom can be used as a backup with any type of data-recording system. We recommend that recorders not use the stored-waypoint capability in a GPS receiver, writing only the waypoint numbers into the log and then later matching log information to GPS data by waypoint numbers or times. In our experience, this procedure is even more prone to errors than fully manual datalogging.

Accurate measurement of the right-angle distance to each sighting is necessary during both strip-transect (at least until enough data have been collected to define a strip width precisely) and line-transect surveys. One can wait for a sighting that is first seen ahead of the aircraft to come perpendicular to the aircraft and then measure the right-angle distance directly. Alternatively, one can measure the radial distance and angle from the flight track when the animal is first sighted and then calculate the right-angle distance to the animal using the high school trigonometry you thought you would never need.

Distance can be measured in several ways. Many investigators place calibrated markings on aircraft windows, wing struts, or other structures and use them to interpolate distances or classify sightings into distance intervals (e.g., Kenney and Scott 1981). This method is rapid and easy, but it generally lacks precision. For example, variation in the position of the observer's head when classifying sighting distance can be a significant source of error. We strongly recommend that observers find a way to standardize their head positions when estimating distance. One can fly over and mark the exact position of each sighting and then calculate the right-angle distance, but this can be very time consuming in areas with many sightings. It is a good method, however, for confirming the accuracy of right-angle distances obtained by other methods and for training observers in estimating distances to fixed objects (e.g., navigation buoys). Another common method for measuring the distance is to measure the vertical angle (the angle downward below horizontal) to a sighting using a clinometer and later on to calculate the distance trigonometrically. This method works relatively quickly, with practice, and is relatively inexpensive. Dodd and Mackinnon (2002) used an electronic digital protractor—a high-tech and much more expensive version of the clinometer—attached to a laser rifle scope. The standard method for estimating distance during shipboard surveys of whales and other marine mammals is to use binoculars with a built-in vertical scale, or reticle (e.g., Thompson and Hiby 1985). We doubt that that this would be useful for aerial surveys of turtles; in our experience, locating a turtle with binoculars at typical aerial-survey speeds can be quite difficult.

The details of survey design depend on many factors. The boundaries of a survey area are usually predetermined based on overall study objectives. Transect spacing represents a compromise between the level of coverage desired (e.g., a 220-m ESW and 10-km spacing between lines result in 4.4% effective coverage of an area) and the available budget for flight time. It may be necessary to conduct a statistical power analysis to determine the level of coverage necessary to achieve a desired level of precision in the estimates. The length of transect that can be sampled in 1 day is determined by aircraft speed, fuel capacity, and endurance; crew endurance; and lengths of transits between survey areas and airports. That length in turn will determine the number of days necessary to complete one sample of the study area and, therefore, the number of replicate samples possible given the available resources.

Interruptions of flights for fuel, food, restrooms, and rest (fatigue avoidance) are frequently desirable, especially with small aircraft lacking the options of rotating positions or taking a break. However, we caution against always breaking for lunch at midday and possibly missing optimal survey conditions. Shoop et al. (1981) and Shoop and Kenney (1992) reported a tendency for rates of turtle sighting to be higher at midday, although they were unable to determine whether this pattern was due to within-day variability in activity cycles or to increased visibility of submerged turtles with higher sun angles and deeper light penetration.

The choice of survey altitude may depend upon the target species. The CETAP surveys, which were designed to sample across taxonomic and size spectra—from large whales down to harbor porpoises and sea turtles—were carried out at an intermediate altitude of 750 ft (230 m; Scott and Gilbert 1982). Fritts et al. (1983) also flew at 750 ft for their multispecies surveys. A survey targeting large whales specifically and collecting sea turtle data secondarily might use a higher altitude; some right whale surveys off the east coast of the United States are currently being flown at 1,000 ft (Slay et al. 2002). The optimum altitude range for sea turtles is 400 to 500 ft (120–150 m; Bayliss 1986; Schroeder and Thompson 1987; Barlow et al. 1988; Marsh and Saalfeld 1989; Marsh and Sinclair 1989).

A survey design must make allowances for inappropriate weather. Sea state, sun glare, and cloud cover have all been shown to affect turtle sightings (Shoop et al. 1981; Scott and Gilbert 1982; Bayliss 1986; Marsh and Saalfeld 1989; Marsh and Sinclair 1989). Barlow et al. (1988) found that the optimal conditions for avoiding biased estimates from aerial surveys for harbor porpoises were cloud cover of less than 25 percent and mirror-like (Beaufort 0) or ripple-only (B 1) sea states, conditions that were present on 5 to 10 percent of flight days (not including days when inclement weather precluded flying at all). For an effective dedicated sea turtle aerial survey, wind and sea conditions should not exceed Beaufort 2 (4–6 knot winds, waves to 1 ft/0.3 m with occasional whitecaps; Bowditch 1966). The larger the overall study area, the easier it is on any given day to select a sampling area with good weather conditions. Planning should include a review of weather statistics for the area and determination of the average proportion of days expected to have suitable visibility and wind conditions, while realizing that extremes are possible (the probability of extended periods of horrible weather is inversely proportional to the flexibility in the aircraft schedule).

AIRCRAFT

For nesting beach surveys, a single-engine aircraft is adequate. It must have a high-wing design to provide adequate visibility for the observer. We strongly recommend that twin-engine aircraft be used for over-water surveys, particularly given the low flying altitudes. A perfect survey aircraft would have at least two engines; the capability of flying safely at low altitudes and slow speeds; a glass bubble in the nose for unobstructed views forward, down, and to both sides; bubble windows on both sides for the second observer team; adequate space for the data recorder and backup observers; a restroom; and fuel capacity for a full survey day with safety reserves. No such aircraft exists, and no researcher could afford to charter it if it did. The Lockheed P-2 "Neptune," a former navy patrol plane, comes closest, but to our knowledge it has not been used for survey work in recent decades. Twin-engine aircraft that have been used for aerial surveys over the last 30 years include the Cessna 337/O-2 Skymaster, Aero Commander (several twin-engine models), DeHavilland Twin Otter, Beechcraft AT-11, Partenavia 68 (including the "Observer" model with extra front-seat visibility), Grumman Widgeon, Grumman Goose, and Grumman Albatross. All of these aircraft have a high-wing design except the Beechcraft AT-11, a bombardier trainer with a forward observation bubble. The three Grumman airplanes, listed in order of increasing size, are amphibious aircraft.

Airships (blimps) are ideal platforms for aerial surveys, with slow speeds, extremely comfortable conditions, and excellent visibility (Hain 1992). However, slow speeds decrease the distance that can be covered, few airship operators are willing to work at significant distances offshore, and costs are prohibitive (e.g., the traveling crew for an airship the size of one of the familiar Goodyear blimps, including pilots, mechanics, and ground crew, is typically 15 to 20 people, all of whom would have to be supported).

OBSERVER TRAINING

Experienced, well-trained observers are critical for any kind of aerial survey project, even small-scale surveys that do not require species identifications. Simply developing an appropriate search image for detecting a turtle on or below the ocean's surface takes practice. Observers need both classroom training in identification characteristics and field preparation. For nesting surveys, observers should be trained on the ground to identify nesting crawls and differentiate them from false crawls, to distinguish fresh from old crawls, and to recognize differences among species. In our experience, avid birdwatchers are the easiest individuals to train and often are the best observers. Ample time should also be spent on data-collection procedures. Clearly explaining to the field personnel (data recorders, observers, and even pilots) how the data are to be utilized improves data quality in the long term, as does timely, continuous feedback about any problems or errors discovered.

OTHER EQUIPMENT

Observers need little equipment. They should have sunglasses with high-quality polarized lenses to minimize the effects of glare from the water surface when searching for submerged turtles. Good-quality binoculars can also be helpful, although binocular choice is frequently a matter of personal preference.

Data Treatment and Interpretation

NESTING BEACH SURVEYS

At the most basic level, nesting beach surveys identify sites of nesting activity and provide an index to the number of nests for some period prior to the survey. With adequate ground truthing to document the ability of the aerial-survey crew to identify the turtle species present and to differentiate between fresh and old crawls and nesting and false crawls, it is possible to generate appropriate correction factors to use when estimating the number of nests from this index (Shoop et al. 1985; Schroeder and Murphy 1999). With sufficiently frequent surveys over a nesting season, it is possible to estimate the total number of nests. Finally, with good information on within-season and between-year re-nesting in the population, it is possible to estimate the total number of nesting females.

OVER-WATER SURVEYS

The most basic data that result from aerial surveys are geographic distributions—locations of sightings plotted on a map, sometimes partitioned by time (month, season, year). Geographic Information System (GIS) software, which is increasingly available and sophisticated, includes a wide range of tools for spatial analyses beyond simple mapping. For example, one can explore the relationships of turtle distributions to a suite of environmental parameters. At any level, however, from simple dots on a map to the most sophisticated multivariate spatial analysis, one must be aware of the potential for significant bias from the distribution of survey effort (see below).

A primary objective of strip-transect or line-transect surveys is estimation of the abundance of the population (or that portion of the population inhabiting the study area). Program DISTANCE (Laake et al. 1993) is the standard software used for complete analysis of line-transect survey data, and it is freely available online (http://www.ruwpa.st-and.ac.uk/distance/). Nevertheless, given the mathematical complexities of abundance estimation, it may be advisable to have a qualified statistician as a collaborator for any major aerial-survey project. From the survey data alone, DISTANCE analysis results in an estimate of minimum abundance, which does not account for turtles that were submerged or simply missed. With adequate data on observer efficiency, the estimates can include a $g(0)$ [detection probability] correction. With adequate data on diving behavior, they can also be corrected for availability. It is possible to apply $g(0)$ and/or dive corrections generated from other studies; however, this is most defensible when the study area and population characteristics are most similar.

Data from aerial surveys that are not conducted with adequate methodological rigor cannot be used to estimate abundance but can be used to develop indices of relative abundance. At its simplest, particularly for a survey that is repeated frequently, the number of animal sightings comprises an index of abundance that can be compared between surveys. The utility of this type of index is improved by standardization—for example, only using data from surveys within defined limits for weather conditions. Standardizing the sightings into a rate per unit of survey, either time or distance (e.g., turtles sighted/h, nests/km of beach), produces a more rigorous index, and again, filtering for weather conditions also improves precision. We have used this sort of distance-standardized *sightings per unit effort (SPUE) index* to combine data from transect surveys and from a variety of platforms-of-opportunity surveys into broader spatial and temporal distributional analyses (Shoop and Kenney 1992). Any surveys where adequate data are collected along the flight paths under appropriate sighting conditions can be incorporated. The data are partitioned into a regular grid of spatial cells, with the grid size determined by the intensity of survey coverage available—from 10-min latitude-longitude cells in broad-scale analyses (e.g., Shoop and Kenney 1992), to as fine as 3-min cells in smaller areas with intensive survey coverage (e.g., Nichols et al. 2008). Because of the spatial component of the SPUE data, they can be incorporated into GIS analysis and modeling, with the bias from uneven distribution of survey effort removed.

Time-series data such as nesting indices, abundance estimates, or relative abundance indices, can be tested to see whether they exhibit significant trends. The usual statistical analyses include linear regression (Gerrodette and Brandon 2000) or analysis of covariance (Forney et al. 1991). It may be advisable to conduct a statistical power analysis to determine how long a time-series is necessary to detect a trend of known magnitude, given the observed level of variability in the survey estimates (Gerrodette and Brandon 2000; see also Gerrodette 1987, 1993).

Special Considerations

Flying over the ocean at low altitudes in a small airplane is dangerous. While this contribution was being prepared, an aircraft crashed off Amelia Island, Florida, during a right whale survey, killing all four people on board. Safety must always be the paramount concern of everyone involved in a survey. The project leader and pilot must be aware of other aircraft that may be operating in or near the study area, as well as banner towers, sightseers along the beach, traffic areas around airports, offshore military operating areas, and other surveys. Pilot qualifications must be impeccable. Timothy Flynn, Aero-Marine Surveys, Inc., a pilot with whom we flew for many years, often said, “The number one qualification for a safe pilot is a wide yellow streak down his back.” Aircraft maintenance and inspection schedules need to be followed meticulously. All personnel involved in the operation should be fully trained in safe operating procedures around the aircraft on the ground and in the aircraft during flight. Everyone should also be fully trained and drilled in emergency procedures. Given adequate resources, some degree of flight training for the observers might be considered. Because aerial turtle surveys can only be conducted in good weather, the safety problems often associated with flying in bad weather are largely avoided, but they should not be ignored. All possible safety and emergency equipment should be provided, including but not limited to fire extinguishers, first aid kits, life raft and survival gear, exposure suits, life vests (inflatables are best given the limited space), emergency radios (ELT in the aircraft, EPIRB with the raft, portable EPIRB, handheld marine VHF), and so forth.

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CHAPTER FOURTEEN

Parametric Analysis of Reptile Biodiversity Data

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and ANTONIO W. SALAS

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Introduction

The analysis of biodiversity data is rarely straightforward, often *ad hoc,* and always challenging. In that respect, any chapter that describes techniques of analysis is necessarily incomplete. In this chapter we broadly outline the ideas and concepts that are important to consider when planning, initiating, and conducting studies designed to measure biodiversity. Beyond definition and description, we also include an extensive literature review as a starting point for interested investigators who wish to pursue a topic in greater detail. We begin with a discussion of biodiversity and its measurement, including richness indices, evenness indices, diversity indices, and rank-abundance models. We follow this with discussions of species-accumulation curves, rarefaction, and the measurement of taxonomic and phylogenetic diversities, and then conclude with a review of selected software packages potentially useful for the practitioner. Where appropriate, we provide opinions and caveats.

Biodiversity Measures

A review of the literature on the concept of biodiversity reveals a bewildering array of explanations and representations of the term, with little agreement on what should be included in the definition. Gaston (1996) began his response to the "What is Biodiversity?" question with the words "bandwagon, buzzword, growth industry, global resource, issue, and phenomenon." He went on to provide a selection of definitions found in the literature. We can, perhaps, agree that *biodiversity* is a measure of the "variety" of entities on earth. What goes into "variety," however, is left up to the individual researcher. Most biologists would likely agree that biodiversity should encompass the concept of total number of species and their respective frequencies of occurrence. One might also include the concept of multiple levels of diversity, for example, taxonomic, genetic, population, and ecosystem (Sandlund et al.1992). Given that biodiversity is a *construct,* or a collective representation of ideas, a suitable definition is not available in all circumstances. We can, however, conceptualize what we mean by "biodiversity" in the context of each particular research question.

Although biodiversity is difficult to define, it can be quantified if the researcher provides an *ad hoc* definition within the context of the given inventory, monitoring project, or research question. In some instances, for example, it may be represented simply by a species-frequency histogram or a list of species counts. In other circumstances, a mathematical expression combining species numbers and geographic distributions will be appropriate (e.g., estimation of density within a given geographical unit). Williams and Humphries (1996) suggested that when biodiversity is measured, an intrinsic, philosophical value of worth is placed on that measurement. Hence, prior to measurement and quantification of diversity, the important questions justifying a particular study should be examined. These questions are, necessarily, study specific.

Below we discuss a selection of potentially useful biodiversity measures, including richness indices, evenness indices, diversity indices, and abundance. We begin with a brief discussion of key concepts related to biodiversity and then revisit each concept to provide specific recommendations for its application, with examples from the literature. We refer readers interested in discussions more detailed than those presented here to the texts by Magurran (1988), Forey et al. (1994), Krebs (1989), and Southwood and Henderson (2000).

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TABLE 23

Mathematical Formulae for Traditional Diversity, Richness, and Evenness Indices

Index Name	*Analytical Formula*[a]	*Reference(s)*
Diversity Indices		
Simpson's	$\frac{1}{\sum_i \left(\frac{N_i}{N}\right)^2}$	Simpson 1949
Shannon-Weaver	$-\sum_i \frac{N_i}{N} \log \frac{N_i}{N}$	Shannon and Weaver 1949
McIntosh's	$\frac{N - \sum_i N_i^2}{N - \sqrt{N}}$	McIntosh 1967
Brillouin's	$\frac{1}{N} \log \frac{N_i!}{\prod_i N_i!}$	Brillouin 1962
Probability of interspecies encounter	$\frac{N}{N-1}\left(1 - \sum_i \left(\frac{N_i}{N}\right)^2\right)$	Hurlbert 1971
Berger-Parker	$\frac{N_{\max}}{N}$	Berger and Parker 1970
Richness Indices		
Margalef's	$\frac{S-1}{\log(N)}$	Clifford and Stephenson 1975
Menhinick's	$\frac{S}{\sqrt{N}}$	Whittaker 1972
Evenness Indices		
Simpson's I	$\frac{1}{S\sum_i \left(\frac{N_i}{N}\right)^2}$	Pielou 1969; Begon et al. 1990
Shannon-Weaver	$\frac{-\sum \frac{N_i}{N} \log \frac{N_i}{N}}{\log(S)}$	Pielou 1969; Begon et al. 1990
McIntosh's	$\frac{N - \sum_i N_i^2}{N - \frac{N}{\sqrt{S}}}$	Pielou 1969
Brillouin's	$\frac{\frac{1}{N} \log \frac{N!}{\prod_i N_i!}}{\frac{1}{N} \log \frac{N!}{\left(\left[\frac{N}{S}\right]!\right)^{S-\left(N-S\left[{}^{N}\!/\!_{S}\right]\right)} \left[\left(\frac{N}{S}+1\right)!\right]^{N-S\left[{}^{N}\!/\!_{S}\right]}}}$	Pielou 1969; Magurran 1988

[a] N_i = number of individuals in the ith species ($i = 1, 2, \ldots, s$); $N_{\max}$ = number of individuals in the most abundant species recorded; N = total number of individuals recorded $(N = \sum_i N_i)$; S = total number of species recorded.

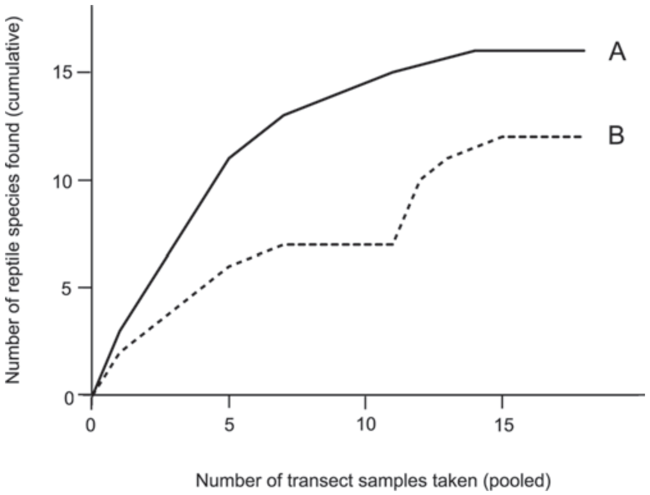

FIGURE 92 Sample species-accumulation curves for measuring reptile species richness at two field sites using transect samples. (A) Standard accumulation curve: increasing sampling effort by adding additional transects leads to an increase in the total number of species collected. The curve reaches an asymptote at approximately 15 species; 10 to12 transect samples would have been adequate to reach this asymptote. (B) Accumulation curve showing the influence of sample order. An initial asymptote is reached at approximately five transects; continued sampling shows that additional species are added with additional transects. This may be caused by the clumping of certain species, changing field personnel, seasonal behavior of certain species, or a variety of other factors. Under the assumption that the samples are fairly homogenous, a randomization procedure to generate plotted means might be a better choice for this site.

Species Richness

Species richness is simply the number of species present in a community (Begon et al. 1990). The utility of this measure is limited in that investigators rely on samples from communities, which they extrapolate to the entire community rather than censusing (actually identifying and counting every individual). Although samples are designed to represent communities as a whole, it is impossible to know how exhaustive or representative a data set really is. We can use simple *species-accumulation curves* (see "Species-Accumulation Curves," below) to obtain an idea of how the number of species being collected is related to the sampling effort and, therefore, to determine when it may be advisable to cease sampling because continued effort provides little additional return (Fig. 92). Often, however, efforts are not great enough to record all species in a given area or community, although complete species-level counts may be possible for some particular reptile groups (e.g., chelonians, crocodilians).

Investigators, conservationists, and politicians often wish to compare levels of biodiversity across communities. Using species richness as the criterion variable is sometimes inadvisable, because the underlying assumptions that sample areas, timing of sampling, and sampling efforts are roughly equivalent in the different communities have not been met. Investigators get around this problem by using methods such as rarefaction and diversity ordering (see "Species Evenness," below), but additional research on methodologies for comparing richness measures is needed. Some analytical methods are available for calculating richness indices (Table 23), and tests for statistical differences between or among communities based on these measures could be designed. Researchers must be sure, however, that tests do not violate the underlying assumptions of the statistical test (e.g., normality and homogeneous variability among communities).

Species Evenness

Species evenness (or *equitability*) is a measure that describes the uniformity with which individuals are distributed among species. Suppose, for example, that five lizard species are represented in a sample. Knowing that 10 individuals of each species are present leads to an interpretation of the community biodiversity different from the interpretation resulting from knowing that 46 individuals of one species are present, but only 1 individual each of the other species. We can express evenness mathematically by dividing a diversity index (see "Species Diversity," below) for a particular community by the maximum possible value it would have if all of the individuals were equally distributed among all species (Table 23).

Species Diversity

Species diversity is a measure that takes into account both species richness and species evenness. Whittaker (1972) suggested a useful classification of diversity, as follows: (1) *α-diversity*—local diversity, as within a habitat or community; (2) *β-diversity*—diversity (species composition) along an environmental gradient (and/or rate of change along that gradient), reflecting the presence of multiple communities along the gradient; and (3) *γ-diversity*—regional species diversity (a combination of α-diversity and β-diversity concepts). Southwood and Henderson (2000) discussed these concepts in detail and present methods for assessing and measuring them.

Hurlbert (1971) referred to species diversity as a "nonconcept." Although we do not fully agree with him, much can be said for this label because the literature contains multitudinous definitions and interpretations of diversity. Investigators must keep in mind that reporting a list of diversity indices from various communities is singularly uninformative unless certain assumptions about the sampling procedures have been met (see "Species Richness," above). Hypothesis testing involving diversity indices can be highly questionable because a number of confounding and extraneous variables associated with data collection make comparisons of two or more indices risky.

In Table 23 we list a variety of common diversity indices that have been used by ecologists. Although the indices may be useful in some studies, use of only a single number (the "index") derived from a set of raw data hides much of the story (much like reporting a mean without also reporting its associated variance and data distribution). Renyi (1961) discussed an entropy-based diversity ordering index, and Hayek (1994b) provided an extensive review of measures of association, both of which may be useful for comparing communities. We suggest that lists of species and numbers of individuals in each be reported rather than (or, at least, in addition to)

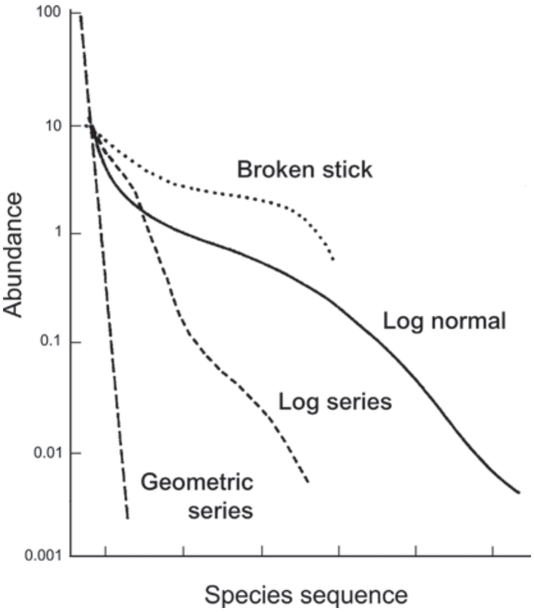

FIGURE 93 Plots showing the theoretical shapes of four common models used to describe rank-abundance patterns. In this diagram, abundance is log-scaled and is plotted against species rank (sequence). Descriptions and mathematical expressions for these models are given in the text. (From Magurran 1988; © A. E. Magurran, reprinted with permission.)

a single index. Having these data available will facilitate discussion, strengthen support for the ecological implications of the data, and reveal the limitations inherent in comparing communities with a single index.

Relative Taxonomic Diversity

For taxonomists, the most important type of diversity is generally α-diversity, that is, local, or within-habitat, diversity. Taxonomic diversity is often used somewhat synonymously with species richness (see "Taxonomic Diversity," below), with the unit of measure being the species and the data collected consisting of a list of those species. Such information is of primary importance for faunal surveys particularly if the following data are also available: (1) species composition and the associated distribution of each (e.g., for studies of biogeography; see Gaston and Williams 1996), (2) extent of current versus historic ranges (e.g., for studies of historical biogeography), (3) relationships between species compositions and environmental variables (e.g., for ecological biogeography), and/or (4) any information potentially useful for conservation and/or management programs.

For some faunistic and biogeographic studies, an index, or metric, of richness or diversity is sufficient for comparative purposes, although the investigator must acknowledge the potential short-comings of this simplified approach (see "Species Richness," above). Comparing species' lists is superior because it allows both common and rare species to be examined if samples have been taken in the same way and at the same scale across habitats or communities (Brown and Lomolino 1998). Surveyors employing appropriate experimental designs can obtain information for delineating biogeographic patterns (e.g., along elevational or latitudinal gradients) and revealing seasonal or community differences. Bannikov (1958), for example, examined the decrease in diversity of snakes in Eurasia along a west-to-east transect using species lists he accumulated, Scherbak (1986) analyzed herpetogeographic regionalization in the Palearctic region, and several authors have compared lizard communities (e.g., Cogger 1984; Kulikova et al. 1984; Orlova and Semenov 1986; Pianka 1986; Ananjeva 1997; Ananjeva et al. 1997).

Species Abundance

Species-abundance models (e.g., rank-abundance or species-abundance diagrams) are models used to describe the relationships between numbers of species and the abundance of each in a particular sampling area. They incorporate all of the information gathered from a community inventory. Consequently, they are more "complete" mathematically than a simple richness index (May 1975; Magurran 1988). Rank-abundance diagrams are constructed by plotting species rank (i.e., based on proportional abundance) on the abscissa and the respective proportional abundance (log-scaled) of each species, ranked from most to least abundant, on the ordinate. The proportion that each species contributes to the total is calculated by dividing the number of individuals in the *i*th species by the total number in all species combined (i.e., N_i / N). The species are then ranked by proportion and plotted as described above. Four models are used most commonly to describe the diversity of species in a rank-abundance plot of a sample (Magurran 1988): the geometric series, the log series, the log normal, and the broken stick (Fig. 93).

GEOMETRIC SERIES

In the *geometric-series model*, the most common species contributes the greatest fraction of the total number of individuals of all species combined, the second most common species a lesser proportion, and so on until all species in the sample have been included (Begon et al. 1990). This model is based on the "*niche-preemption hypothesis*" (*sensu* Magurran 1988) under the assumption that the most abundant species preempts the greatest proportion of resources in an area, the second most abundant species preempts the second greatest proportion of resources in an area, and so on. Hence, the rank abundances of species in a community, when ordered from the most abundant to the least abundant, produce a straight line when plotted (Fig. 93). The rank abundance of the *i*th ($i = 1, 2, \ldots, S$) species (N_i) as a function of niche preemption and total number of species is given by

$$N_i = \frac{Nk(1-k)^{i-1}}{1-(1-k)^S} \qquad (1)$$

where N = the total number of individuals; k = the niche-preemption parameter, which is generally estimated as the fraction of the total number of species over the total number of individuals, although newer methods for iteratively estimating k from sample data have been developed by Caruso and Migliorini (2006) and He and Tang (2008); and S = the number of species (May 1975). Field research by Whittaker (1977) suggested that this model is useful in species-poor areas or in early successional communities (see Magurran 1988) where the niche-preemption hypothesis would suggest that the first species to arrive in an area will use the largest fraction of the available resources, the second species to arrive will use the *k*-fraction of the remainder, and so on.

LOG SERIES

The *log-series model* is closely related to the geometric series (May 1975) and may represent the logical progression of a generalized successional community through time (Fig. 93; Magurran 1988). Mathematically, the log series can be expressed using a constant "a" as a model constraint (defined in eq. 4 below), and an "x" that represents the number of species predicted to have one individual. The series then progresses such that $ax^2/2$ = the number of species expected to have two individuals . . . and ax^n/n = the number of species expected to have n individuals. We can obtain the total number of species (S) by'

$$S = a[-\log(1-x)] \tag{2}$$

where x is obtained from an iterative solution of

$$\frac{S}{N} = \frac{1-x}{x[-\log(1-x)]} \tag{3}$$

such that

$$a = \frac{N(1-x)}{x} \tag{4}$$

with N defined as the total number of individuals of all species present (Pielou 1969; Magurran 1988). In practice, the expected number of individuals can be calculated using the relations above, and these expected values can then be compared to the observed number using a likelihood ratio, chi-square, or related statistic (Magurran 1988; Sokal and Rohlf 1995).

LOGNORMAL SERIES

Many species' abundances in natural communities display a *lognormal distribution* (Fig. 93; May 1975; Sugihara 1980; Magurran 1988). This distribution is discussed in May (1975) and Gray (1987). The mathematical representation of the lognormal model is quite simple, with the distribution of the abundance classes represented by a histogram showing the numbers of species (y-axis) containing various numbers of individuals (x-axis; e.g., 4 species contain 3 individuals, 7 species contain 5 individuals, etc.). The equation can be written as

$$S(R) = S_o e^{(-2\sigma^2 R^2)} \tag{5}$$

(Pielou 1969) where S_o is the number of species in the modal class, σ^2 = variance of the lognormal distribution (i.e., a measure of the width of the distribution) calculated from the histogram, and R is the number of species in an individual class; therefore, $S(R)$ = the number of species in a given class (R) to the left and right of the modal class (S_o). The observed distribution can be compared to a theoretical, expected distribution based on, for example, perfect normality around the modal class. Often, however, this distribution does not adequately represent species in the tails of the distribution because of sampling bias. Pielou (1975) showed how a truncated lognormal curve can be used to estimate the parameters of the equation when the relative abundance of very rare species produces a histogram with no left tail.

BROKEN-STICK SERIES

This model was first proposed by MacArthur (1957) to represent how individual species in a sample contribute to the total number of species assuming nonoverlapping niches with abundance proportional to the size of the niche (Pielou 1969; Magurran 1988). In the *broken-stick series* model, which has been referred to as a "biologically realistic expression of the uniform distribution" (Magurran 1988, p. 29), each species contributes a proportion to the whole such that the graph represents a stick that has been broken into random fragments and laid out by ranking the size of the fragments (Fig. 93; Begon et al. 1990). The mathematical representation for this model (May 1975) is

$$S(n) = \frac{S(S-1)}{N}\left(1-\frac{n}{N}\right)^{S-2} \tag{6}$$

where $S(n)$ = the number of species in the abundance class containing n individuals, with N defined as the total number of individuals across all abundance classes, and S defined as the number of species observed. As before, expected and observed distributions can be compared to test the fit of this model. Magurran (1988) reviewed the literature concerning use of this model for many communities.

Species Density and Continuously Distributed Data

Types of Data

Variables that are measured in ecological studies can be classified in a variety of ways. It is important that variable type be kept in mind at every stage of an investigation (design, data collection, database management, analysis, and reporting) so that the appropriate type of data (e.g. continuous, categorical, etc.) will be collected to answer a specific research question. Often when designing studies, field biologists must balance resource (e.g., time, personnel, funds) availability against research needs (such as accuracy or precision) and must determine which data are needed to answer the questions at hand and how they should be gathered. Inasmuch as appropriate analyses require appropriate data, we briefly describe data types and general statistical considerations. Following the classification scheme of Sokal and Rohlf (1995), variables can be grouped into three broad categories: measurement variables, ranked variables, and categorical variables.

MEASUREMENT VARIABLES

A *measurement variable* is a variable based on a quantitative measurement. Investigators can determine whether differences between these variables (e.g., mean values for different populations or the same population at different times), expressed as numbers, are significant statistically. Measurement variables fall into two broad data categories: continuous and discrete. *Continuous variables* are those in which a theoretically infinite number of values can fall between any two numbers, although in practice, instrumentation (the capability of a measuring device to measure smaller and smaller amounts) limits the realized continuity of any distribution (Hayek 1994b; Sokal and Rohlf 1995). *Discrete variables,* or *meristic variables,* have only fixed values. We may, for example, count ventral scales and record numbers such as 100 or 122, but not a number such as 100.115, which has no meaning. Measurement variables are quite useful in a statistical sense, as many established distributions (and tests derived from them) such as the normal, exponential, or gamma distributions for continuous variables, and the binomial, geometric, and Poisson distributions for discrete variables can be used to analyze these quantitative measurements.

RANKED VARIABLES

These variables, which are not necessarily based on measurements, represent an order or magnitude (Sokal and Rohlf 1995). Imagine that we record the order of emergence for five Copperhead (*Agkistrodon contortrix*) from their hibernaculum. We may record these as 1, 2, . . . , 5 (first, second, . . . , fifth), but unlike with discrete variables, we are not implying that the difference between adjacent variables (e.g., 1 and 2) is the same in magnitude as the difference between any other two adjacent variables (e.g., 2 and 3); snake 2 may have emerged 1 hour after snake 1, but 3 days before snake 3. Another way to consider ranked variables is in the context of nonparametric statistics. For example, after measuring the snout-vent lengths (SVL) of female *Thamnophis* from two populations, one may wish to compare their mean lengths. If preliminary testing reveals that we cannot meet the assumptions of normality and equality of variances for a two-sample *t*-test, the data may be ranked and then analyzed with a nonparametric Wilcoxon two-sample test (Sokal and Rohlf 1995). In this example, because the test is based on the absolute *ranks* of the data, we lose information about the absolute magnitude of difference between any two measurements.

CATEGORICAL (ATTRIBUTE) VARIABLES

Categorical variables are variables used to describe qualitative data. We might record *Varanus* females as “gravid” or “not gravid” or *Phrynosoma* females as “pre-reproductive,” “reproductive,” or “post-reproductive.” Several statistical techniques have been designed especially for use with categorical variables (e.g., logistic regression and contingency table analysis); they are discussed in Agresti (1990) and Hosmer and Lemeshow (2000). Measurement variables are often grouped for convenience into categories (such as “docile” or “aggressive” or “very aggressive”), but that does not imply that the data are necessarily categorical in nature; rather, it may indicate that the researcher simply did not wish to divide the data any further (e.g. “somewhat aggressive” or “extremely aggressive”).

Considering the way data are categorized is useful from a statistical design and analysis standpoint, because such categorizations dictate the type of analyses that can be done. Investigators often use a variety of univariate and multivariate statistical techniques. One of the most common violations of statistical assumptions seen in the literature is the failure to test for normality of the data (e.g., by comparing the empirical data that were collected to a theoretical distribution that is normal, using a Komolgorov-Smirnov or Shapiro-Wilk statistic), an underlying assumption of many well-known techniques. We strongly urge all scientists to seek the advice of a qualified statistician or biometrician prior to collecting data. These professionals can assist researchers in determining which variables to include in sampling and how to analyze them appropriately.

Species Density

DEFINITIONS

Species density is a common measure of biodiversity that refers either to the number of individuals of a single species or to the number of species present in an area during a given time or sampling effort (Hayek 1994b). *Population density* is a measure of the total number of individuals per unit area, whereas *relative density* is a measure of the densities of a number of populations relative to each other (Lancia et al. 1994). Another term common in the reptile literature is *linear density,* a measure of population density along a linear distance rather than an areal extent (King 1986; Parker and Plummer 1987; Cross 1998). Measures of linear density are particularly useful for aquatic and semiaquatic species (e.g., crocodilians), in which numbers per unit of linear shoreline may provide a more appropriate expression of the data.

ESTIMATES AND STATISTICAL CONSIDERATIONS

Estimating density using estimates of population size is not always straightforward, because the *effective area* (Lancia et al. 1994) and the area to which the density estimate is to be applied may not be equivalent, which leads to a biased estimate (White et al. 1982; Anderson et al. 1983). As an example, if one wishes to estimate the density of a species over 1 km^2, but does this with 10 subsamples of 10 m^2, then the effective area of the sample is the sum of the 10 subsamples (100 m^2) and not the desired 1 km^2. However, setting up a proper sampling design generally allows one to extrapolate over larger areas if the subsamples are, in fact, representative of the entire area of interest. If the area sampled is known (or assumed) to include the entire area of interest, then the estimate of population density, *D,* and its accompanying variance are given by

$$\hat{D} = \frac{\hat{N}}{A} \qquad Var(\hat{D}) = \frac{Var(\hat{N})}{A^2} \tag{7, 8}$$

(Lancia et al. 1994) where A = area, $\hat{N}$ = estimate of abundance, and Var($\hat{N}$) = estimated variance of the abundance estimate. If we assume that density is relatively constant over the length of a linear transect (e.g., shoreline), then we can calculate linear density by replacing length for area in the above equations. Because we can calculate both a point estimate (i.e., $\hat{D}$) and a variance, it is possible to test for a significant difference from a known (perhaps historic) density distribution under the assumption of normality (Sokal and Rohlf 1995). Also, relative density distributions from two areas or two years can be compared using distributional statistics or nonparametric statistics. For a thorough review of field investigational techniques, including methods of determining density and abundance, see Skalski and Robson (1992).

Density can also be estimated using distance sampling. The primary sources of information on use of this technique are Burnham et al. (1980) and Buckland et al. (1993). *Line-transect sampling* (i.e., traversing a transect and measuring the distance and direction to a visible species of interest) and *point-transect sampling* (i.e., standing at a point and observing distance and direction to a visible species of interest) are commonly used to estimate bird and mammal densities (Buckland et al. 1993). The investigator establishes line or point transects through a study area and then estimates distances from a known point on the transect to any animals that are detected. These distances are then used to construct a detection function (i.e., the probability of detecting an organism that is present at a given distance) for adjusting density estimates (see "Transect Surveys, Including Line Distance," in Chapter 13). Area is estimated as twice the transect width (to account for left and right sides of the transect) multiplied by the transect length for line transects and by π times the square of the radius for point transects. Abundance is simply the number of animals encountered during the survey (assuming all animals were detected). Density is estimated by substituting these values into equations (7) and (8) for each point or line transect in the survey. Much has been published on the adjustment of estimates of animal detectability and errors in distance estimates (Burnham et al. 1980; Seber 1986; Buckland et al. 1993). These methods are not widely used by reptile biologists, perhaps because of the inherent difficulty of detecting some animals. Anderson et al. (2001) tested this methodology on *Gopherus agassizii* populations in the Mojave Desert. They discovered that even novice field personnel can adequately use transect sampling to estimate abundances, and they concluded that transect sampling should be considered for large-scale monitoring projects.

Species-Accumulation Curves

A *species-accumulation*, or *"collector's" curve*, is a relatively straightforward method for estimating local species richness (Fig. 92). The curve is constructed by plotting the cumulative number of species found as a function of the sampling effort (Soberón and Llorente 1993). Sampling effort is often represented by the number of samples collected, but many other measures, such as trap days, quadrat area, length of drift fence, and so forth, can also be used (Colwell and Coddington 1995). Species-area curves, wherein the total number of species is plotted against the size of the sampling area, are reviewed briefly in Hayek (1994b).

To optimize sampling effort, one can plot the number of species collected against the number of samples taken; when an "eye-ball" examination indicates that an asymptote has been reached, the investigator can stop sampling because the likelihood that additional species will be encountered is very small. However, if one wishes to compare estimates of species richness across sites or at one site over years, a more complex methodology must be used to find the maximum number of species expected—that is, the asymptotic maximum of the accumulation curve. Once a best-fit curve is fitted through the empirical data, then one can use iterative approaches to estimate the maximum (or supremum) of the curve; if known statistical distributions are used to plot the curve, then basic calculus can be used to find the maximum value the curve will attain. Many methods exist to find a best-fit curve; the program EstimateS is quite useful in this regard (see Appendix II).

The order in which samples are added to a survey can affect the shape of its accumulation curve; changes in the curve can be related to sampling error, amount of effort expended, or heterogeneity among sampling units (Fig. 92; Colwell and Coddington 1995). Randomizing the sampling order (e.g., of transects or sampling points; Colwell and Coddinton 1995) or using subsamples of the measure of effort (Holdridge et al. 1971) will help to control this problem when estimating average richness. The mean numbers of species calculated from the subsamples can then be plotted to construct a curve that is less affected by sample order; in addition, variances and confidence intervals can be constructed at each point along the curve.

Several models have been developed for fitting curves to accumulation plots in order to estimate an asymptotic maximum number of species. An early effort by de Caprariis et al. (1976) is equivalent to the *enzyme-kinetics model* of Michaelis-Menten in which the rate of substrate conversion (= rate of encountering additional species) is related to the concentration of the substrate (the number of species present). Much research on the statistical properties of this estimator can be found in the literature; we refer interested readers to reviews by Raaijmakers (1987) and Colwell and Coddington (1995). Another important model is the *negative-exponential model,* which estimates the probability that an additional individual will be a new species by assuming a linear dependence on the current species list (Miller and Wiegert 1989; Soberón and Llorente 1993). This probability approaches zero as the asymptote of the accumulation is reached, thus providing a common-sense interpretation of the model (Colwell and Coddington 1995). A third set of models, the *nonasymptotic models* (Colwell and Coddington 1995; reviewed in Palmer 1990), includes the *log-linear model* (accumulation increases with the log of effort) and the *log-log model* (equivalent to MacArthur and Wilson's 1967 island biogeographic species-area curve). These are only a few of the most common curve-fitting techniques. For a review of both parametric and non-parametric models for fitting curves to accumulation plots, see Colwell and Coddington (1995).

The most useful information provided by curve-fitting models is often an estimate of how richness is likely to change as a function of increased effort (Colwell and Coddington 1995). Also, one can compare species-accumulation curves (e.g., rate of increase) across time or space, or construct tests of point estimates (e.g., maximum richness) using a variety of goodness-of-fit statistics (Sokal and Rohlf 1995). Simply plotting maximum richness against time for a long-term study area may also be informative, as it can provide information about community stability and resilience, particularly if the community has been affected by a known event (e.g., fire, flood, introduction of an invasive species).

Rarefaction

A goal of many studies is to compare the species richness of different areas or of a single area at different times. Given that species richness generally increases with the number of samples taken, up to a theoretical asymptote (Sanders 1968; Hurlbert 1971), the sample sizes of the entities being compared must be statistically equitable (Sanders 1968; Hurlbert 1971; Peet 1974; Heck et al. 1975).

Sanders (1968) devised a simple method to reduce samples to a common size before comparing them. He ranked the species in a sample relative to their representations in the sample, and calculated cumulative percentages. He then took random sub-samples of size n from the total pool of original samples taken (total of N), developing new species-accumulation plots, or *rarefaction plots*. The mean number of species found from these repeated subsamples was then plotted as a function of sub-sample size (Sanders 1968; Simberloff 1972; Hayek 1994b). An obvious drawback to this method is that it lacks a probabilistic framework (Hayek 1994b) and is strongly affected by sampling methodology (Fager 1972). Simberloff (1972, p. 417) suggested that "[n]ot only is the rarefaction method incorrect, the degree to which it is incorrect is markedly dependent on sample size." Sanders's (1968) original method consistently overestimated the expected number of species ($E(S_n$) drawn randomly from a collection of N individuals and S species (Hurlbert 1971; Heck et al. 1975; Hayek 1994b), because the correct estimate of the number of species should be based on a hypergeometric probability distribution (Simberloff 1972; Heck et al. 1975) rather than on the *ad hoc* method he used. Based on the hypergeometric distribution Hurlbert (1971) gave the correct function for estimating $E(S_n)$:

$$E(S_n) = S - \sum_{i=1}^{S} \left[\frac{\binom{N-N_i}{n}}{\binom{N}{n}} \right] \tag{9}$$

where, $E(S_n)$ = expected number of species in the sample of n individuals that are randomly selected from a total of N individuals, S = the total number of species, and N_i = the number of individuals of the ith species in the unrarefied sample. Heck et al. (1975) provided a formula for calculating the variance associated with this estimate ($V(S_n$); owing to the complexity of the expression, we refer interested readers to the original source.

If one samples with replacement instead of without replacement (i.e., the hypergeometric distribution), then a multinomial distribution can be used; Heck et al. (1975) provided the computational formula for $E(S_n)$:

$$E(S_n) = S - \sum_{i=1}^{S} \left(1 - \frac{N_i}{N}\right)^n \tag{10}$$

Again, the variance formula for $V(S_n)$ is quite complex and can be found in the original paper. The authors also provide a useful example of how to use $E(S_n)$ to estimate sufficient sample sizes for valid comparisons (i.e., simply solve for n in the equation and select a sample size which provides a desired proportion of the total number of species [S] to be taken).

When using a rarefaction technique, one must keep in mind the relationship between the population and the sample (i.e., the "parent population" and the "collections;" see Hayek 1994b). For the rarefaction methodology to be useful, samples must be collected at random. Given the habitat specificity of reptiles (and hence the sampling strategies used to collect them), sampling sites are often clustered in specific habitats rather than randomly distributed (Reinert 1993); therefore, rarefaction procedures can lead to biased results (Hayek 1994b). Nevertheless, rarefaction methods have been found to be particularly useful when sizes of species assemblages differ (Buzas 1979; Hayek 1994b), so long as sampling is random. This method can be particularly useful for comparing areas or time periods if samples were collected in a similar fashion (i.e., in the same season, same habitats, etc; Hayek 1994b).

Taxonomic and Phylogenetic Diversity

Taxonomic and phylogenetic diversity have become the vanguard of research in conservation, in which identification of areas with the greatest numbers of species available for protection is often a goal. In large part this reflects the many advances in techniques for elucidating phylogenetic relationships and species clustering patterns, and the development of diversity measures that take advantage of this new information. Interested readers should consult the excellent book by Forey et al. (1994) on this topic.

Taxonomic Diversity

Often, the only data that are available for groups of organisms is some measure of group membership based on taxonomic patterns. Consequently, investigators have developed measures of diversity based on the information inherent in classification branching points and nodes (Vane-Wright et al.1991, 1994; Williams and Humphreys 1996).

ROOT WEIGHT

In the *root-weight method* each species in a classification is weighted based on its distance from the root of the classification. Essentially, the number of branching points between the root and each of the species present in the classification is counted. Weights are then determined by dividing the total node count of the classification by the node count for each species; thus, species closest to the root node have the highest weights. These weights values are summed to provide a diversity score for a given biota (Vane-Wright et al. 1991).

HIGHER-TAXON RICHNESS

This method for calculating taxonomic diversity is an extension of the root-weighting procedure that gives greater weights to higher taxa (i.e., taxa farther from the initial branching node in the taxonomic tree) regardless of the number of species that each may include (Williams et al. 1991, 1993). For this measure, species are compared in pairwise fashion. In biotas with large numbers of higher taxa, the divergences of species tend to be closer to the root of the classification than in biotas with smaller numbers of higher taxa; thus, the latter biotas will have lower node counts. Again, investigators can convert taxonomic branches to ratio scores as above (counting the number of branching points between the root of the classification and each species and then dividing the total node count of the classification by the node count for each species); the sum of these weights for a given classification is its diversity score (Williams and Humphreys 1994).

SPANNING-SUBTREE LENGTH

This methodology is based on a measure of the total amount of a classification that is represented in a given biota and, hence, is related to a common measure of phylogenetic diversity (Faith 1994; Williams and Humphreys 1994). In this case, the intervening nodes along a cladistic path are counted as a surrogate for path length, although the validity of this methodology has been strongly questioned (Faith 1994). These node counts are then converted to percentages of the total score for the entire classification. An extension of this method takes into account the actual cladistic divergence between species in a given biota to account for particularly evenly distributed species ("cladistic dispersion"; Williams and Humphreys 1994).

Phylogenetic Diversity

Measures of phylogenetic diversity were developed to introduce ideas and results from systematics into a conservation framework (e.g., see Engstrom et al. 2002 for work on endangered turtles). These measures are calculated using a mathematical function of the number of lineages implied by a phylogenetic tree, in order to discern the total amount of a classification that is represented by a given taxon or biota.

Faith (1994, p. 50) considered *phylogenetic diversity* to be a subset of species diversity and defined it as the "sum of the lengths of those branches from the estimated phylogeny that are spanned by the species subset." Say, for example, that one wishes to increase the phylogenetic diversity of a protected reserve. A subset of taxa on the preserve has a given phylogenetic diversity, and hence a logical question would then be "How much more diversity would be gained by introducing another taxon?" Species that are phylogenetically distinct should offer the largest contribution to the overall diversity of any biota. Hence, changes in branch length gained through adding an additional taxon provide a straight-forward approach for examining this question. Faith (1992a, 1992b, 1994) provided a variety of formulae for calculating these measures.

Molecular Advances

Recent advances in biochemical and molecular methods for determining phylogenetic relationships have provided new information that has changed our understanding of the phylogenies of several squamate groups, for example, the agamids and gekkonids of the arid Palearctic region (Macey et al. 1998, 1999, 2000) and the geckoes of Kazakhstan and northeastern China (Macey et al. 1997, 2000). A particularly useful method is *flow cytometry* in which cells (or chromosomes) suspended in a fluid medium are passed through a beam of light. The particles cause the light to scatter and may fluoresce. Detectors pick up and analyze the fluorescent emissions and the deflected and transmitted light, determining physical and chemical properties of the particles and sorting them. Several thousand particles per second can be analyzed and sorted with this technique. In addition, it does not require that specimens be sacrificed, making it especially useful for the study of rare or endangered species. It also reveals complexes of cryptic species. Data obtained with this technique can be presented as a phylogenetic tree or cladogram. Murphy et al. (1997) have outlined its use in biodiversity surveys. Also, see MacCulloch et al. (1996), who used this technique in their studies of *Anolis* lizards.

Reconstructive Biogeography

Reconstructive biogeography is a term used to describe the process of reconstructing taxonomies based on either morphological or genetic patterns (i.e., phylogeography) under the premise that contemporary geographic distributions of species are a function of historic processes or events related to population bottlenecks, immigration/emigration, and population expansion/contraction. Most often, both current molecular techniques and biogeographical histories are used simultaneously to construct species' origins and spread over time (for example, with poison frog species; Santos et al. 2009). Similar techniques are used to investigate fossil faunal assemblages (Smith 1994). In this instance, the number of taxa that could occupy the "morphospace" of a given size category is estimated based on the existing fossil record for a given geographic area. The occurrence of forms is recorded, and formal morphological character analysis is used to construct phylogenetic trees that permit the description of phylogenetic diversity in recent as well as fossil reptiles (Rasskin-Gutman and Buscalioni 2001; Ciampaglio et al. 2001; O'Keefe 2002).

Making Inferences Based on Monitoring Data

Making valid inferences from monitoring data depends on a number of factors, including the following:

1. *Project design*. Few things are more frustrating to a biometrician than being asked to consult on a project after the data have been collected only to find that the study was so poorly designed as to preclude any valid conclusions. All studies, particularly those that will involve monitoring over time, require specific statistical designs; otherwise, complex questions cannot be answered nor can practical management decisions be made.
2. *Scale*. The scale of a given habitat (e.g., microhabitat vs. macrohabitat) can be an exceedingly important issue to consider when designing a biodiversity study, particularly if target species are using available habitat resources at different scales seasonally or ontogenetically. Consulting the literature and other experts in the field is paramount to establishing the best sampling regime.
3. *Spatiotemporal considerations*. Monitoring data are inherently temporal, because a major goal of any monitoring project is to detect change (if any) over time. Thus, knowing when to sample so that the data collected are relevant to the research questions being asked is particularly important. If forecasting or time-series analyses (analyses of data, of which time is a dominant variable, taken at specified time intervals) are to be used, sampling periods must be equally spaced, a requirement that must be accommodated in the research plan (Bowerman and O'Connell 1993;

Fuller 1996). Relatively few ecologists consider the spatial nature (through space or time) of sampling, automatically assuming that all replicates are valid if they are carried out at different locations. The possibility of nonindependent samples (e.g., spatial correlations) cannot be ignored, however, and independence of different locations must be tested before data can be used as replicates in analyses. All ecologists involved in analyzing data from field studies should become familiar with the basics of spatial statistics theory (e.g., Isaaks and Srivastava 1989; Goovaerts 1997) and application (e.g., Fortin and Dale 2005).

4. *Statistical analyses and interpretations.* We highly recommend that investigators seek advice from a statistician familiar with ecological sampling designs and analyses before initiating a study. In addition, reports and publications must be written in such a way that even readers who are not well versed in statistics will understand that statistical significance does not necessarily mean that the results of a study contain useful biological information. Conversely, managers, journal editors, and others must recognize that the absence of statistical significance does not mean that a study and the data it encompasses are not important to the issue at hand. All researchers must be honest in their data gathering and reporting, even if a study does not warrant a peer-reviewed article. Additionally, researchers must be cautious when extrapolating beyond the limits of their data, which can be extraordinarily misleading and, in the worst case, provide impetus for short-sighted management decisions.
5. *Database management.* Heyer et al. (1994b), in their recommendations for amphibian-monitoring studies, suggested that monitoring data need to be stored, managed, and shared. We fully agree and strongly urge everyone involved in monitoring to store their data in a consistent way and to include metadata that describe the study, sampling techniques, and sampling locations in detail. This will allow future researchers to design comparative studies for use in potential meta-analyses. Without consistency over time and place, biodiversity monitoring may provide very little comparable information.

Analyzing Biodiversity Data

Biodiversity data should be analyzed using known, validated techniques. We do not mean to suggest that the development of new techniques is unimportant. Rather, adherence to methodologies that can be replicated and that are valid for answering specific research questions should be employed so that collected data are consistent and comparable over time and space.

Certain minimum data should be gathered and maintained as part of all monitoring studies. These include dates; exact location(s), preferably including corrected GPS coordinates; times of sampling; descriptions of techniques used to acquire data; and statistical sampling design. In many cases, it will be necessary to preserve voucher specimens, which must be adequately prepared, labeled, and stored so as to ensure their long term utility and availability for later verification of identification and research (see Chapter 6, "Voucher Specimens"). Noss (1990) has discussed the use of indicators for monitoring biodiversity.

We do not recommend any minimal set of statistical (or other) analyses that must be carried out on all data sets. The key is to make all data available in a usable format so that they can be easily retrieved and used over time. Individuals designing and carrying out monitoring (or other) studies will identify their own research questions and sets of pertinent analyses. Again, we urge the use of known, replicable techniques selected with the assistance (advice) of a statistician.

Computer Programs for Analyses of Biodiversity Data

Below we list selected computer packages that we have found to be useful for analyses of biodiversity data. The list is not exhaustive. We recommend these programs only because we have found them useful; we do not endorse their use or imply their superiority to other programs.

Biodiversity Analyses

BIOTA

Biota is an extremely flexible database management package (Colwell 2007). It is especially useful for managing collections as well as records and images.

ESTIMATES

Investigators can use this program (Colwell 2009) to compute randomized accumulation curves, a variety of diversity indices, and indices of similarity between samples, as well as to estimate richness.

RAMAS

This modeling program (Applied Biomathematics 2007) is useful at the landscape scale. It can be used to build predictive models of extinction, population growth, and other population parameters. It is quite useful for predicting trends through time based on vital rates.

WORLDMAP

This user-friendly software package (Williams 2001) is designed to explore geographic patterns in diversity at any spatial scale. It has been used extensively for assessing taxonomic diversity (e.g., Loiselle et al. 2003; Manne and Williams 2003).

Taxonomy and Phylogenetics

DELTA SYSTEM

Delta System is an integrated set of programs for formatting and managing taxonomic databases for use by other programs (Dallwitz 1980).

CHAPTER FIFTEEN

Population Size and Demographics

GORDON H. RODDA

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Introduction

In this chapter, I address the problem of estimating reptile density, that is, the number of individuals of a given species that are present in a given area. Chapter 14, in contrast, tackles the composition of reptile assemblages, or the number of species present and their identities. Biological diversity is generally judged in terms of both quantities. For example, to compute a Shannon-Weiner biodiversity index one needs a list of the species present and an estimate of the relative density of each.

Key Definitions

Many researchers and research teams use technical terms differently. To avoid confusion, I define the terms that I will use, below. Terms that are specific to particular population sampling methods are defined in the relevant sections.

Abundance is the total number of individuals in a population. For example, at a specific time there might be 4,112,378 Brown Water Snakes (*Nerodia taxispilota*) in the United States. This is the formal definition of abundance; informally, abundance is often used to connote density. When one informally describes a particular species as *abundant,* the implication is that the species is exceptionally numerous per unit area. The number of individuals per unit of space is *density.* Routinely distinguishing density from abundance is of great value. Density is typically expressed as the number of individuals per hectare (e.g., 41 Brown Water Snakes/ha). A rare (= low-density) species can have a high abundance if it occurs over a large area, whereas a high-density species can have low abundance if it is restricted to a small area (its geographic range is very small).

Although "abundance" has a strict formal definition, the term is often loosely applied to any measure of a species' commonness. For example, range size, occupancy (presence/absence), relative density (= relative abundance), and absolute population density are all metrics of abundance. *Range* or *range size* is overall extent, usually expressed as the number of hectares (or square kilometers) that are inhabited by a species or population. Range size may be monitored to provide an indirect measure of abundance, assuming that if the species' range were to increase, such expansion would likely reflect increasing abundance (conversely, a range contraction would likely indicate a numerical decline). *Occupancy* is the proportion of a specified area that is inhabited; for example, 23 percent of a county might be inhabited by the Common Gartersnake (*Thamnophis sirtalis*). This is often called the *Proportion of Area Occupied,* or *PAO.* PAO usually refers to a local area, whereas range size usually characterizes the entire range of a species, although either can be applied at any scale. Like range size, occupancy is also monitored in order to provide an indirect measure of abundance (MacKenzie and Nichols 2004), this time assuming that if a species' occupancy rate were to increase, such increase would reflect increasing abundance, and vice versa.

Logically, a true relative density would be a focal species' density expressed as a percentage of a reference density, such as that of the most common species in the area. For example, Gopher Snakes (*Pituophis catenifer*) might have a relative density of 54 percent compared to gartersnakes in a particular area. Unfortunately, this is not how the expression "relative density" is conventionally used. *Relative density* (less correctly *relative abundance*) is a capture rate expressed with the conviction that higher animal densities are reflected in higher capture rates. For example, in a forested habitat one might catch 12 Eastern Gartersnakes (*Thamnophis s. sirtalis*) in four hours, whereas in adjacent riparian habitats the yield is 43. This common usage of capture rate is that of an index. An *index* is some measure that does not quantify the exact parameter of interest (in our case, density), but rather is believed to be correlated with the feature of interest. We expect

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that capture rate and density are correlated. Capture rates often are expressed in terms of number of detections per unit of searching or trapping effort, typically *Catch Per Unit Effort,* abbreviated CPUE. *Detection* is the term statisticians apply to any contact with a focal organism, whether a capture, a sighting, or merely proof that the individual passed by (e.g., a fecal sample). The above example of 43 riparian gartersnakes detected in 4 hours is a CPUE, usually expressed as (43 snakes/4 h =) 10.75 snakes per hour. This same relative density could be expressed in terms of other capture rates. For example, if those 43 snakes had been sighted along 2 km of river, one might express the capture rate as 21.5 snakes per km. The danger in reporting a capture rate per unit area is that one might be misled into confusing a capture rate with an absolute population density. An *absolute population density* is the total number of individuals in an area expressed as individuals per unit area, typically individuals per hectare or square kilometer (potentially individuals per km for species living in linear habitats such as those bordering rivers). A relative population density equals an absolute population density only if one has detected 100 percent of the population in the area surveyed. How likely is that?

The difference between relative abundance and absolute population density is mirrored in the distinction between a survey and a census. A *population survey* is an effort to obtain a representative sample of a population in a specific area; a *census* is a count of all individuals in a specified area. In general, very small plots can be censused, but large study areas must be surveyed. If 10 percent of the individuals in a specified area were counted in a survey, the survey's *detection fraction* would be 10 percent, or 0.1. Alternatively, one could state that the species' *detectability* per individual in this system was 0.1. Suppose that our detection of 43 water snakes in 2 km of stream represented a 10 percent detection fraction. In that case the relative abundance (capture rate from our survey) would be 21.5 snakes per km, but the absolute population density (from a theoretical census) would be 215 snakes per km. In the older literature the word "census" is often treated as synonymous with survey, but the distinction is useful and increasingly observed.

Species richness, species presence, range, occupancy, relative density, and absolute abundance are all examples of *state variables.* They denote which characteristic (state) of the ecological system is of interest. Each of these may be of interest in its own right, or it may be used as an index of some other feature. For example, occupancy is sometimes used as an index of abundance, and absolute population density is often used as an index of habitat quality. Some researchers have quantified the morphological symmetry of individual animals (termed "*fluctuating asymmetry*"), based on the observation that populations under stress exhibit a greater proportion of asymmetric individuals (Sarre 1996). In this example, fluctuating asymmetry is used as an index of population stress, another possible state variable. It is often helpful to list the state variable(s) of interest and to determine whether the feature will be estimated directly or through a more readily measured index.

A variable of interest is often contrasted with a nuisance variable. A *nuisance variable* is something that one needs in order to get where one is going, but that is not in itself of interest. For example, detectability is often quantified as a first step for estimating abundance from capture rates; for researchers using this approach, abundance is the parameter of interest, and detectability is the nuisance variable needed to estimate abundance. For other researchers, detectability may be of interest, and abundance could be a nuisance parameter influencing detection rates.

All measures of abundance are statements about the condition of a population, not about an individual. Thus a population biology statement must be made with reference to a population, which must be sampled. Unless one is counting every individual of an extremely rare species (Whooping Cranes [*Grus americana*]), one must sample. Defining the population and obtaining a statistically valid sample of it are thus the keys to estimating abundance.

Population is a hodgepodge term connoting a pool of individuals of one species occupying a common space. Typically, a population under study includes just those animals that are present within a specified geographic area, often a study plot or land under single ownership. Defining a "study population" in this manner is convenient, but it is arbitrary (animals do not stop at property lines) and has no biological basis. A biological population is the pool of all individuals that interbreed; for specificity, I call this a *reproductive population.* It is often useful to contrast the bounds of a study population with the boundaries of the population to which inferences from the study should apply.

Chapter Organization

Population biology—and this chapter—is more than the measurement of density or abundance. Survival, fecundity, dispersal, immigration, and other demographic[1] characteristics of a population may be of importance. Furthermore, there is no one correct way to estimate abundance; understanding the advantages and disadvantages of different methods as well as likely sources of error is the key to a wise decision. Some of the potential sources of error are best illustrated by a short story, followed by an analysis of key strategic issues:

Haldre is a biologist who works for Art, the Refuge Manager at the Elk Mountain National Wildlife Refuge. Art is worried about the Blue-tongued Mango Skink (Hiaasen 1991), which is found primarily in wet meadows on the refuge and in a few surrounding areas. The skink may be suffering from competition with the introduced Wolf Skink, which prefers drier, more open sites. Under political pressure from local businesses, Art allows logging operations on the refuge. By removing the trees, logging incidentally creates more habitat for Wolf Skinks. Art is worried about the impact this may be having on the Blue-tongued Mango Skinks. Art summons the refuge biologist, and asks, "How many blue-tongues do we have?" With uplifted palms, Haldre shrugs. Art tries a new tack, "Are there more or fewer than in years past?" "We've never had the money to conduct surveys, so I can't tell you," Haldre snaps. Art retrenches, "What do you need to conduct surveys in both wet meadows and clear-cuts?" Haldre thinks for a moment and answers, "Technician time, vehicles, permission, 54 pitfall buckets, and 120 m of drift fence material." Art grants her wish and sends her forth.

After 3 months, Haldre returns for a meeting with Art. She reports, "We found 36 Blue-tongued Mango Skinks and

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[1] The Greek roots of the word "demography" indicate that it is the study of human populations, but by extension the word has come to include the analysis of populations of any species.

11 Wolf Skinks in the wet meadows. We found 19 blue-tongues and 110 Wolf Skinks in the clear-cuts." Art asks if the surveys went according to plan. "Well," Haldre notes, "we ran into a lot of rocks when digging the pitfalls in the clear-cuts." "Did those arrays have fewer skinks?" Art asks. Haldre notes, "It is a little hard to tell, as some of those arrays were placed in ravines, whereas other arrays were in flatter areas. We had somewhat fewer Wolf Skinks in the arrays placed in the more level sites, but I'm not sure if the difference was real or due to some combination of habitat, substrate, and slope characteristics." Art has trouble digesting that complexity, so he moves on to his main question, "Well, are there more or fewer mango skinks in the clear-cuts than in the wet meadows?"

Haldre begins, "We counted fewer Blue-tongued Mango Skinks in the clear-cuts than in the wet meadows, but we don't know if the density of these skinks was lower in the clear-cuts." Art jumps in, "How can that be? If you caught fewer, there must be fewer!" Haldre reflects, "Pitfalls measure some mixture of activity level, movement patterns, and density. When the skinks move more often or cover greater distances, they fall into pitfalls more frequently. Without an independent measure of activity, we are on shaky ground inferring density. We did not measure activity or density by itself in either habitat; therefore, we can't say if our counts correlate with densities. If the blue-tongues move less in drier areas or more exposed sites than they do in moister or less exposed areas, or if because of dryness they stay closer to their home holes in the clear-cuts, they would be caught less-often in the clear cuts, although their population density could be equivalent or greater. Moreover," Haldre goes on, "the apparently greater density of Wolf Skinks in the clear-cuts may be inhibiting the movements of the blue-tongues, leading them to be trapped less often than they would be otherwise. Using the same protocol does not ensure that the same fraction of the population of each species will be detected."

"Chalupa!" Art exclaims in frustration, "these trap counts are maddeningly difficult to interpret; let's go to an index of abundance that is independent of the movement rate of the skinks." "Okay," Haldre replies, "What if I organize a series of visual surveys to count the number of individuals of each species sighted on the ground along transects that are placed randomly in the two habitats? There will be additional costs for construction of the transect trails and, because these animals are active at night, for overtime and headlamps." Art slumps in frustration, but concedes, "Well, if that is what it takes . . ."

By now it is autumn, and Haldre gears up for visual surveys the following spring. In July she comes back to Art with some preliminary data, "Art, the average sighting rates for Blue-tongued Mango Skinks in wet meadows and clear-cuts were 0.48/km and 0.33/km, respectively; those for Wolf Skinks were 0.02/km and 1.02/km. The difference in Wolf Skink numbers is statistically significant." "So," Art interprets, "the density of mango skinks does not differ between habitat types, yet a lot more Wolf Skinks were found in the clear-cuts than in the wet meadows." "Actually," Haldre notes, "the failure to find a statistical difference between the index values for the blue-tongues does not mean the sites are the same; it means only that the difference, if any, was too small to detect with the sample size available. With a bigger sample size, we might find a statistically significant difference."

Art, glowering at the technicality, continues: "So, for mango skinks the density difference between the two habitats is small or negligible." "Actually," Haldre interjects, "the difference may not be small, and it could be in the opposite direction. The density of the blue-tongues in the clear-cuts could be higher, based on what we know. If we assume that the fraction of the blue-tongue population that was detected in wet meadows was the same as the fraction detected in clear-cuts, and if we suppose that the correlation between the sighting rate and true density is excellent (say, $r^2 > 0.8$), then the chance that the true density of Blue-tongued Mango Skinks in clear-cuts was higher would still be substantial. A modest difference in counts, correlated moderately well with the phenomenon of interest, leaves open the possibility of contrary results. Unfortunately, we have no data on either the assumption of equal detectability or the strength of the correlation between that relative measure and the absolute abundance. Correlations between sighting rates and true densities may be excellent, but they are often poor and sometimes abysmal. Furthermore, my impression is that because of the denser vegetation in wet meadows, the meadow surveys quantified the skink population in a smaller strip of land than did the surveys in the clear-cuts. The area actually being surveyed may not be the same in the two habitats. Even if the counts were the same, the densities might differ. Another way to think of it is that a greater percentage of the blue-tongue population in wet meadows may be impossible to see."

Art is intrigued by this line of reasoning, "How can we measure the percentage of things that we cannot see?" Haldre is well versed in sampling methodologies and therefore has a succinct answer, "There are three approaches for estimating the fraction of animals that are overlooked:

1. Census small statistically representative areas, extrapolate to the entire study area, and infer the overlooked fraction from the proportion of area not censused (e.g., distance sampling).
2. Sample the same site repeatedly, ignoring animals recorded in earlier samples; the rate of appearance of new sightings extrapolated to an infinite number of sighting events indicates the number of animals overlooked previously (e.g., depletion or removal sampling).
3. Mark a known number of animals and search for the marked animals repeatedly to determine what fraction of them is overlooked in each subsequent pass (e.g., mark-recapture sampling).

Each method has unique assumptions and challenges, but also a means of estimating the detection fraction and, therefore, absolute population density. By quantifying detectability, absolute population estimators produce results that are independent of the sampling conditions. Absolute population density estimates are ideal for long-term monitoring because they do not require constancy or equivalence of searchers, species, sighting conditions, weather, season, and so forth. In addition, each provides a density that can be referenced to a habitat type and land area for estimation of habitat use and total population size."

"Which of these absolute population density estimation techniques would be best for skinks?" Art asks. "That depends," Haldre muses, "on the nature of the species and the habitat. For example, if marking is traumatic for these small lizards and causes them to hide, a technique such as depletion/removal sampling may be best, because it does not require marked animals. Depletion/removal sampling is, however, very sensitive to movements of skinks in and out of the study plots, and it requires that they be captured. Removal/depletion sampling tends to be grossly imprecise if the detection fraction is low. These animals are small and cryptic, making distance sampling a long shot in heavy vegetation, although it is more promising if conducted on recently cleared land. If the lizards do not grossly alter their behavior in response to marking, mark-recapture might be a good choice for either habitat. A preliminary study of several approaches would help clear up this uncertainty." Art wholeheartedly agrees, knowing that he has enough money remaining in his budget this fiscal year to fund a preliminary study, and the full survey costs will fall on next year's budget, by which time he will have transferred to a new refuge.

Art's successor, Cy, summons Haldre the following year and asks for an update. Cy explains that his priorities are a little different from Art's. "The refuge," Cy explains, "is being sued by an environmental group that wants to stop the logging because of its potential impacts on the Blue-tongued Mango Skinks. Furthermore, the pet trade has taken a sudden interest in this animal with the intriguing name, and the refuge is losing skinks to collectors who especially seek out the brilliantly colored juveniles. Other skinks are occasionally run over on the refuge's roads. What can you tell me about the population's responses to the threats? What will happen to the skink population if we do nothing? What will happen to the skink population if we stop the logging? Do we need to restrict driving on the refuge at night when the skinks are on the roads?" Haldre suggests a trio of approaches: "We could implement a density-monitoring program to determine the absolute size of the local Blue-tongued Mango Skink population and its composition (size/age/sex distribution). Through periodic road surveys, we could quantify the number of animals killed on the roads. These two approaches would enable us to determine the fraction of the population that is killed by traffic. Through mark-recapture we could estimate the survival of each size/age class in order to project population changes and estimate the importance of the various threats. The analysis would use life tables to suggest whether removal of juveniles (by pet collectors) or removal of adults (killed by automobiles) is the greater threat to the health and stability of the population. To address the lawsuit, we could use the life table data to perform a population viability analysis, which, within the accuracy limits of the data, would provide a probability that the population will persist under present management practices. If the analysis indicates that the population is not likely to persist, then we can explore the demographic impacts of alternate management practices, such as a halt to logging or a prohibition of nighttime driving on the refuge. Although these management practices cannot be rigorously evaluated without realistic field experiments, life table–based projections will help to identify strategic demographic quantities to measure and will narrow the range of viable management choices, at relatively little cost."

"Speaking of cost," Cy squirms, "this program you are suggesting sounds like it will cost a bundle." "Yes," Haldre intones. "These questions you are asking are not easily answered."

Key Strategic Issues

DETECTABILITY

The preceding dialogue draws attention to a number of key issues. Foremost is whether to quantify detectability. This issue continues to divide herpetologists, but those in favor of estimating detectability seem to be gaining the upper hand. The traditional approach is to compile simple counts, preferably under standardized conditions, express the counts as mean capture rates, and assume that detectability is constant across the conditions of interest. If detectability is equal across the conditions of interest, the capture rates will be proportional to population density. In this case the capture rates will be an inexpensive, precise, and unbiased index of absolute population density, or simply a good estimate of relative density.

The argument against relying on counts is that without a measure of detection fraction one can never know whether the key assumption—constant detectability—is correct, which is unlikely. Previous studies indicate that the probability that detectability remains constant over any length of time or range of conditions is very low (Whitaker and Shine 1999; Sun et al. 2001; Harvey 2005; Weir et al. 2005; Cherkiss et al. 2006). Species routinely differ in detectability, observers differ, habitats differ, localities differ, competing species change abundance, weather varies, equipment changes, and so forth (Bailey et al. 2004a, 2004b; Rodda, Campbell et al. 2005; Rodda, Dean-Bradley, and Fritts 2005). The collective scope of potential changes in detectability is substantial, often larger than the range of density differences. Species, habitat, and observer differences in detectability can be 10-fold or more (Rodda, Campbell et al. 2005; Rodda, Dean-Bradley, and Fritts 2005). If the data collected are to be used by future researchers, then interpreters of future comparisons will need to assume that the current observers have seen or trapped (i.e., detected) the same fraction of the population that the original observers did, without knowing what that fraction was. Unfortunately, an implied assumption of equality requires that all conditions remain constant, even ones the researcher has not yet noticed. MacKenzie and co-workers (2006, p. 34) provided a lovely example of the importance of estimating detectability in a system that should have been favorable for constancy of detection. The study included just one set of observers, one study species, one season, one major habitat type, and good replication. The study appeared to reveal that antelope in winter benefit most from nearness to water sources—that is, until they factored in detectability. When detectability was integrated into their analysis, they found that slope and sagebrush density were the only factors affecting animal density; nearness to water did not benefit the antelope at all, but only made them easier to detect. Failure to incorporate detectability into an analysis not only weakens the inference but can also lead to erroneous conclusions. For another example, see Box 9, "Relative Abundance in Snakes: A Case Study," below."

Another rationale for relying on counts or indices is that every reasonable method of estimating detectability requires assumptions, some of which are questionable and a potential source of error (Engeman 2003). However, measuring detectability eliminates the need to make the larger, overarching assumption that detectability is constant (Anderson 2003). Although equal detectability may be only one assumption, it is a whopper; the magnitude of the errors that assumptions inherent in estimating detectability might engender are modest in comparison. The researcher's challenge is to assess which set of assumptions is most likely to be problematic and to design a sampling methodology that avoids dubious assumptions. In that spirit, we can focus on assumptions and their plausibility.

BOX 9 RELATIVE ABUNDANCE IN SNAKES: A CASE STUDY

David A. Steen, Craig Guyer, and Lora L. Smith

For cryptic reptiles such as snakes, counts generated by inventory and monitoring projects are often used to assess relative abundances among habitats (e.g., Todd and Andrews 2008) or over time (e.g., Winne et al. 2007). Such information can then be used to evaluate habitat suitability and populations trends. Field researchers often assume that *detection probabilities* (i.e., the probability of detecting a species when it is present; see "Key Definitions" in Chapter 15) equal 1.0 in all situations, or they attempt to satisfy that assumption by replicating treatments and increasing sampling effort (Mazerolle et al. 2007). However, detection probabilities likely vary considerably, particularly in relation to habitat characteristics (MacKenzie et al. 2006). Thus, the number of individuals of a species recorded can be influenced by how detectable that species is in different situations. Because detection probabilities for a species are rarely the same among sites, differences in numbers of animals observed may be due to inequities in detection rather than to differences in population size (MacKenzie and Kendall 2002), which makes comparisons among populations difficult. To obtain the most accurate measures of relative abundance, investigators need to calculate detection probabilities for the populations they are investigating and incorporate them into their measures of abundance. It is likely that researchers who do not account for detection probability are simply evaluating their own abilities to observe individuals (MacKenzie 2005b).

Recent developments in models of abundance that allow for imperfect detection have enabled researchers to estimate a species' abundance without marking individual animals (Royle and Nichols 2003; Royle 2004b). This improvement overcomes some of the logistical problems associated with sampling cryptic animals. In particular, Royle and Nichols (2003) have shown how one can estimate abundance by exploiting the relationship between local population size and detection probability. Their models require multiple visits to each site so that detection/nondetection data can be evaluated for each species encountered (MacKenzie et al. 2002).

Here, we illustrate the importance of employing detection probabilities and demonstrate how they can influence abundance estimates. We use the model of Royle and Nichols (2003) to evaluate the efficacy of drift fences and box traps in estimating relative abundances of snakes in three Longleaf Pine (*Pinus palustris*) forests. We assess whether increased pooling of data within a year, increased sampling across years, or increased replication of trap arrays is likely to minimize error in assessing abundance from drift fences and box traps.

Study Sites

We sampled snakes at three sites. The Conecuh National Forest site is located in south-central Alabama, approximately 30 km southwest of Andalusia. The forest covers approximately 33,590 ha, and efforts to restore Longleaf Pine as the dominant tree species are ongoing. A single large drift fence array (see "Funnel Traps, Pitfall Traps, and Drift Fences," in Chapter 5, and "Pitfall-Trap Surveys," in Chapter 13) was constructed in each of 12 sites. Each array consisted of a centrally located square box trap (design adapted from Burgdorf et al. 2005) with four funnel-shaped entrances. A 30-m fence of 0.6-cm-mesh hardware cloth extended outward from each entrance. Funnel or pitfall traps were placed at the end of each fence. For a detailed description of the study site and trapping methodology, see Guyer et al. (2007).

The second site was the Solon Dixon Nature Center, a field station located in south-central Alabama (adjacent to Conecuh National Forest) that is used for forestry education by Auburn University. The property, approximately 2,145 ha, has been subjected to intensive management for timber production. Drift fence arrays were constructed in 15 sites in 2002 as part of another study (Rall 2004). Funnel traps were placed along fences, and bucket traps were placed at the terminus of each fence arm. Centrally located box traps were added in 2003. The study site and trapping methodology are described in detail in Rall (2004).

Our third site was Ichauway (Joseph W. Jones Ecological Research Center), an 11,740-ha tract dominated by Longleaf Pine and managed to maintain the structure of old-growth forest. Ichauway is located in southwestern Georgia, approximately 45 km southwest of Albany. Forestry practices are largely confined to prescribed burning and hardwood removal. Drift fence and box trap arrays (Burgdorf et al. 2005) were constructed in 16 sites associated with a predator exclosure study. An array consisted of four 30-m arms of hardware cloth that converged on funnels opening into a central box trap. For a detailed description of the study site, see Smith et al. (2006).

At all sites, movement of snakes between traps was at least theoretically possible due to the long-distance movement potential of some species (e.g., Steen et al. 2007). However, recaptures at multiple

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traps were so rare that we consider traps to be functionally independent.

Abundance Estimates

We used the Royle method for inferring individual detection probabilities from counts, as implemented in program PRESENCE (version 2.0, Hines 2006; Appendix II), without covariates, to estimate detection probabilities and abundances of 28 snake species found on one or more of the three sites. For the purposes of this exercise, we defined abundance as the number of snakes whose movements bring them into contact with the area where traps were located at a particular site. We restricted our analysis to captures recorded during months that coincided with the active season. We analyzed data collected between April and November 2005 and March and October 2006 at Conecuh; between April and August 2002, 2003, and 2004 at Solon Dixon; and between April and September 2005, March and September 2006, March and October 2007, and April and October 2008 at Ichauway. We pooled captures within each month and used the month as the shortest sampling occasion. A trap array was the spatial unit of replication.

We determined the effects of study duration, temporal pooling of data, and number of traps on abundance estimates, using data from Ichauway, the most intensively sampled site. To examine study duration, we calculated abundance estimates for the four most commonly captured species after 1, 2, 3, and 4 years of sampling. To examine temporal pooling of data, we estimated abundance based on counts pooled from an entire year and compared them with estimates based on data pooled at monthly intervals. This was also done for the four most commonly captured species. To examine the effect of number of traps on estimates of abundance, we calculated abundances for the North American Racer (*Coluber constrictor*) based on counts from 4, 8, 12, or 16 traps selected randomly, using year as the sampling occasion. In each case, statistically significant effects were determined by inspection of 95 percent confidence limits. We expected these intervals to narrow as number of years of sampling increased, as pooling of samples increased, and as number of traps increased.

Results and Discussion

Abundance estimates varied widely among and within species (Table 24). Confidence intervals surrounding abundance estimates were broad, indicating considerable uncertainty in the calculated values. In fact, in only five instances did confidence intervals exclude the possibility that abundance was zero. The four species most commonly captured at Ichauway were the North American Racer, Coachwhip (*Coluber flagellum*), Common Gartersnake (*Thamnophis sirtalis*), and Eastern Hog-nosed Snake (*Heterodon platirhinos*). Increasing study duration did not generate more precise abundance estimates of these four species because confidence limits did not narrow with each additional year (Table 25). In addition, confidence intervals overlapped an abundance of zero for all species and years except the Racers during years 2 and 4 (Table 25). When we calculated abundance estimates using data pooled across monthly and yearly sampling occasions, confidence intervals were wide and overlapped with zero for both iterations for all species except the racers (Table 26). Confidence intervals were wider for data pooled by year than for those pooled by month for all species except the Racers. Finally, for racers, confidence limits of abundance estimates generally decreased as number of traps increased from 4 to 16. Detection probability and abundance-estimate confidence limits excluded a detection probability of zero when all 16 traps were used (Table 27).

Investigators have frequently used count data as a surrogate for relative abundance in studies of snake assemblages. Traditional analyses compare the number of individuals of each snake species to a uniform distribution via a chi-square or equivalent test. Rejection of the null hypothesis is used to infer differences in abundance between groups of individuals. For example, we captured significantly more racers (126) than Coachwhips (50) at Conecuh National Forest ($P < 0.0001$; $\chi^2 = 32.8$, $df = 1$). The temptation is to conclude that the species with more recorded captures is more abundant. However, when adjusted for detection probabilities, confidence intervals surrounding abundance estimates based on these counts did not support that conclusion (estimated abundances and 95 percent confidence intervals were 38.32 and 0.79–75.84, respectively, for racers, and 28.53 and 0.00–57.07 for Coachwhips; Table 24). A difference in the detection probabilities of the two species (Table 24) confounds straightforward interpretation, as it would if comparing one species between two sites.

Despite sampling efforts that included 12 to 16 traps visited over 2 to 4 years and count data pooled across a time interval of 1 year, we generated detection probabilities that consistently included zero for all but one of 28 species sampled. For racers, we generated a reasonable detection probability and estimate of abundance only when we used data from the maximum number of traps (16) and pooled data within a year for each of 4 years. Given the effort and expense of this sampling effort, our results are discouraging for studies in which the main goal is to compare relative abundances via box traps and drift fences.

Our results for racers indicate that count data may provide a reasonable estimate of population size for a

TABLE 24

Snake Species Captured at Three Sites in the Southeastern United States, Their Detection Probabilities, and Abundances[a]

Site	*Observed*	*Detection Probability*	*Standard Error*	*Lower Confidence Interval*	*Upper Confidence Interval*	*Estimated Abundance*	*Standard Error*	*Lower Confidence Interval*	*Upper Confidence Interval*
Conecuh									
Agkistrodon contortrix	153	0.12	0.06	0.01	0.23	36.08	18.75	–0.67	72.82
Agkistrodon piscivorus	5	0.05	0.05	–0.04	0.15	6.03	5.62	–4.99	17.05
Cemophora coccinea	8	0.13	0.07	–0.02	0.27	2.47	1.86	–1.18	6.12
Coluber constrictor	126	0.14	0.07	0.01	0.27	38.32	19.15	0.79	75.84
Coluber flagellum	50	0.08	0.04	0.00	0.16	28.53	14.56	0.00	57.07
Crotalus adamanteus	22	0.01	0.03	–0.06	0.07	237.95	925.90	–1576.82	2052.72
Diadophis punctatus	1	0.05	0.06	–0.07	0.18	1.00	1.52	–1.99	3.98
Heterodon platirhinos	7	0.08	0.05	–0.03	0.18	5.02	3.78	–2.38	12.42
Lampropeltis elapsoides[b]	2	0.00		0.00	0.00	1068.21		–1885.73	4022.15
Nerodia fasciata	13	0.17	0.06	0.04	0.29	3.43	2.04	–0.57	7.42
Opheodrys aestivus	1	0.05	0.06	–0.07	0.18	1.00	1.52	–1.99	3.98
Pantherophis guttatus	9	0.10	0.05	0.00	0.20	5.66	3.39	–0.98	12.30
Pantherophis obsoletus	23	0.04	0.04	–0.04	0.11	37.83	40.54	–41.63	117.28
Pituophis melanoleucus	23	0.02	0.05	–0.08	0.11	87.55	275.38	–452.20	627.29
Regina rigida	2	0.00	0.00	0.00	0.00	1068.21	14712.69	–27768.66	29905.09
Sistrurus miliarius	11	0.14	0.06	0.03	0.25	4.96	2.67	–0.27	10.20
Storeria occipitomaculata	2	0.00	0.00	–0.01	0.01	1068.21	35463.11	–68439.48	70575.91
Tantilla coronata	5	0.05	0.05	–0.04	0.15	6.03	5.64	–5.02	17.09

(*continued*)

TABLE 24 (*continued*)

Site	*Observed*	*Detection Probability*	*Standard Error*	*Lower Confidence Interval*	*Upper Confidence Interval*	*Estimated Abundance*	*Standard Error*	*Lower Confidence Interval*	*Upper Confidence Interval*
Conecuh									
Thamnophis sauritus[b]	2	0.00		0.00	0.00	1068.21		–1888.95	4025.38
Thamnophis sirtalis	33	0.07	0.04	–0.01	0.15	24.53	14.54	–3.97	53.04
Virginia striatula[b]	4	0.00		0.00	0.00	1068.21		–1886.18	4022.61
Virginia valeriae	1	0.05	0.06	–0.07	0.18	1.00	1.52	–1.99	3.98
Ichauway									
Agkistrodon contortrix[b]	52	0.01		–0.06	0.07	202.84		–1881.54	2287.22
Agkistrodon piscivorus[c]	1								
Cemophora coccinea[b]	3								
Coluber constrictor	324	0.06	0.03	0.01	0.12	116.56	56.30	6.21	226.91
Coluber flagellum[b]	177	0.02		–0.33	0.36	446.83		–9971.52	10865.19
Crotalus adamanteus[c]	38								
Crotalus horridus[b]	12	0.00		–0.01	0.01	815.37		–6615.30	8246.03
Farancia abacura[c]	1								
Farancia erytrogramma[c]	1								
Heterodon platirhinos[b]	98	0.00		–0.12	0.13	663.10		–12421.27	13747.46
Heterodon simus[b]	2	0.00		0.00	0.00	1732.75		–3683.21	7148.71
Lampropeltis getula	70	0.04	0.02	0.01	0.08	54.80	23.77	8.22	101.39
Lampropeltis elapsoides[b,c]	3								
Nerodia erythrogaster	11	0.03	0.02	–0.01	0.07	12.99	8.97	–4.59	30.56
Nerodia fasciata[b,c]	13								
Pantherophis guttatus[b]	80	0.01		–0.11	0.13	209.27		–2385.62	2804.16

Pantherophis obsoletus	50	0.02	0.02	–0.02	0.06	73.26	81.71	–86.89	233.41
Pituophis melanoleucus	45	0.00	0.01	–0.01	0.02	477.32	1264.04	–2000.20	2954.84
Thamnophis sirtalis[b]	105	0.08		–0.12	0.14	492.55		–8307.01	9292.11
Solon Dixon									
Agkistrodon contortrix	30	0.08	0.03	0.02	0.14	22.05	9.27	3.88	40.22
Agkistrodon piscivorus	4	0.03	0.04	–0.05	0.11	9.44	13.62	–17.25	36.13
Cemophora coccinea	7	0.02	0.04	–0.05	0.10	21.38	36.70	–50.56	93.31
Coluber constrictor[b]	113	0.04		–0.49	0.58	148.38		–2001.01	2297.77
Coluber flagellum	27	0.01	0.01	–0.02	0.03	363.57	908.06	–1416.22	2143.37
Crotalus adamanteus	9	0.07	0.04	–0.02	0.15	9.32	6.45	–3.33	21.97
Crotalus horridus	4	0.00	0.00	0.00	0.00	2095.25	8988.01	–15521.25	19711.75
Diadophis punctatus	1	0.10	0.09	–0.08	0.27	1.10	1.26	–1.38	3.57
Farancia abacura	4	0.03	0.04	–0.05	0.11	9..44	13.67	–17.36	36.23
Heterodon platirhinos	19	0.00	0.00	0.00	0.00	2215.07	2494.03	–2673.23	7103.37
Lampropeltis elapsoides	1	0.10	0.09	–0.08	0.27	1.10	1.26	–1.38	3.57
Nerodia fasciata[c]	2								
Opheodrys aestivus[c]	3								
Pantherophis guttatus	8	0.01	0.03	–0.04	0.06	42.95	88.24	–129.99	215.89
Pantherophis obsoletus	14	0.04	0.04	–0.03	0.11	21.52	20.65	–18.96	61.99
Pituophis melanoleucus	9	0.01	0.02	–0.04	0.05	118.71	514.20	–889.11	1126.54
Sistrurus miliarius	1	0.10	0.09	–0.08	0.27	1.10	1.26	–1.38	3.57
Storeria occipitomaculata	3	0.09	0.07	–0.04	0.22	2.71	2.15	–1.50	6.93
Tantilla coronata	47	0.02	0.04	–0.05	0.12	82.73	101.39	–115.99	281.45
Thamnophis sirtalis	7	0.02	0.04	–0.05	0.10	21.38	36.85	–50.84	93.60
Virginia valeriae	1	0.10	0.09	–0.08	0.27	1.10	1.26	–1.37	3.57

a. Detection probabilities and abundances estimated using the model in Royle and Nichols (2003); standard error, and lower and upper confidence intervals of detection probabilities on the left, and of abundances on the right.

b. Variance/covariance matrix not computed successfully.

c. Numerical convergence reached with ≤ 3 significant digits.

TABLE 25

Abundance Estimates for the Four Most Commonly Captured Snakes on Ichauway, 2005–2008, for Each Successive Year of Study[a]

Year 1				*Year 2*				*Year 3*				*Year 4*			
Obs[b]	Est. Abun.	Lower CI	Upper CI	Obs.	Est. Abun.	Lower CI	Upper CI	Obs.	Est. Abun.	Lower CI	Upper CI	Obs.	Est. Abun.	Lower CI	Upper CI
Coluber constrictor															
72	56.09	–19.33	131.5	207	53.03	6.54	99.53	252	123.92	–21.76	269.59	324	116.56	6.21	226.91
Coluber flagellum															
62	68.11	–22.96	159.19	102	174.57	–656.59	1005.73	142	59.64	–30.94	150.21	177	446.83[c]	–9971.52	10865.19
Thamnophis sirtalis															
32	238.48	–1694.59	2171.56	80	279.33[c]	–4097.72	4656.38	97	90.31	–37.33	217.95	105	492.55[c]	–8307.01	9292.11
Heterodon platirhinos															
29	347.49	–4516.37	5211.34	68	407.72[c]	–6492.58	7308.02	87	101.25	–41.94	244.44	98	663.1[c]	–12421.3	13747.46

a. Abundance estimates calculated using the model in Royle and Nichols (2003). Captures were pooled within each month, and the month was used as the sampling occasion.

b. Obs. = observed, Est. Abun. = estimated abundance, CI= confidence interval.

c. Variance/covariance matrix not computed successfully.

TABLE 26

Species Detection Probabilities and Abundance Estimates for the Four Species of Snakes Most Frequently Captured at Ichauway, 2005–2008[a]

Species	*Detection Probability*	*Standard Error*	*Lower Confidence Interval*	*Upper Confidence Interval*	*Estimated Abundance*	*Standard Error*	*Lower Confidence Interval*	*Upper Confidence Interval*
Coluber constrictor	0.474	0.2068	0.0686	0.8794	77.26	37.51	3.73	150.78
Coluber flagellum	0.0267	0.2148	–0.3944	0.4477	1176.94	9481.52	–17407	19760.7
Thamnophis sirtalis	0.1198	0.1806	–0.2343	0.4738	155.66	235.67	–306.25	617.58
Heterodon platirhinos	0.0096	0.0489	–0.0861	0.1054	1898.89	9634.06	–16984	20781.6

a. Detection probabilities and abundances estimated using the model in Royle and Nichols (2003). Each year considered a sampling occasion; capture data pooled within each of the four years of study.

TABLE 27

Effect of Trap Number on Detection Probabilities and Total Estimated Abundances of *Coluber constrictor* on Ichauway, 2005–2008[a]

Number of Traps	*Species Detection Probability*	*Standard Error*	*Lower Confidence Interval*	*Upper Confidence Interval*	*Estimated Abundance*	*Standard Error*	*Lower Confidence Interval*	*Upper Confidence Interval*
4	0.023	0.318	–0.6	0.646	482.33	6665.3	–12581.61	13546.28
8	0.3073	0.257	–0.196	0.811	40.08	35	–28.53	108.69
12	0.4183	0.225	–0.022	0.859	56.62	33.13	–8.31	121.55
16	0.474	0.209	0.064	0.884	77.26	37.87	3.03	151.49

a. Data pooled within years and used as the sampling occasion.

species that is trapped frequently, but sampling effort would have to be relatively intensive and sample sizes large. When we compared monthly vs. yearly pools of data, we found that detection probabilities for this species were considerably higher when year was treated as the sampling unit. Thus, an investigator may have more confidence examining trends in abundance on a yearly rather than seasonal basis. If thresholds of sample size and effort exist beyond which one may have high confidence in abundance estimates, we did not reach it after recording 324 captures of racers in 16 traps over 4 years of study at Ichauway.

We are not the first to suggest that cryptic animals defy conventional sampling techniques. Bailey et al. (2004b) suggested that unadjusted counts not be used to compare population sizes of terrestrial salamanders, whose detection probabilities are extremely low and variable. Population size estimators that assumed equal catchability performed poorly for freshwater turtles (Koper and Brooks 1998). Although reptile detection probabilities are often assumed to equal 1 (Mazerolle et al. 2007), we have documented that the probability of detecting cryptic species when they are present is, in fact, extremely low and the abundance values uncertain.

Several lines of reasoning suggest, however, that our estimates of detectability and abundance can be refined. For many species, we were unable to obtain model convergence and nonsensical results were often produced. This may be a function of us using default settings in program PRESENCE, but low sample sizes limit our options in any case. The Royle and Nichols (2003) model assumes that error variance conforms either to a Poisson or a negative binomial distribution; whether these distributions are appropriate

(continued)

(continued)

for snake populations is unknown. In addition, snakes are most susceptible to capture within box traps when they are most active. However, activity is not static across a year. We attempted to minimize this bias by not considering data generated during winter months, when snake activity is low. However, even within an active season, some species exhibit bimodal activity patterns, wherein activity peaks in the spring and again in the fall (Gibbons and Semlitsch 1987). A decrease in capture rates at any time will decrease detection probabilities and inflate confidence intervals. This suggests estimates may be refined by conducting intensive sampling efforts over a shorter time period that more accurately coincides with the highest activity period for a particular snake species. Finally, we did not integrate any covariates (e.g., percent canopy cover) that might affect detection probability and, therefore, our ability to improve the precision with which abundance might be estimated. Such variables are known to lead to improved precision in estimates of abundance (Royle and Nichols 2003).

Snakes have become more popular as study organisms in ecology (Shine and Bonnet 2000), and studies of population trends among different species assemblages or sites is increasingly common (e.g., Krysko 2002; Russell, Hanlin et al. 2002; Renken et al. 2004; Willson et al. 2006; Winne et al. 2007; Cagle 2008; Owens et al. 2008; Ruthven et al. 2008; Todd and Andrews 2008). Development of efficient and productive snake traps (Burgdorf et al. 2005) represented a great stride forward in efforts to sample snakes in terrestrial systems. Yet when the goal is to quantify relative abundances, our analyses indicate the need either (1) to explore additional novel sampling techniques (e.g., Rodda, Fritts, Clark, et al. 1999; Cablk and Heaton 2006) that will increase detection rates above those using current methods or (2) increase sampling effort above what is typical for most snake studies.

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While investigators generally concede that quantifying detectability is desirable, it is not always practical. Real-world problems such as the lack of a usable marking technique, high population turnover, and impracticality of obtaining a sufficient sample may preclude the use of certain techniques, no matter how desirable. Before plunging into the details of various analytical techniques, we should review some of the key strategic issues in choosing a population-sampling protocol. Among the key considerations are (1) whether or not individuals can be individually marked and/or cohort marked, (2) the scale of time and space over which the size and composition of the population remain closed (geographic and demographic closure), and (3) the degree to which detectability or any other key attribute of the population is heterogeneous.

MARKING

A key issue in determining which abundance-state variables an investigator can monitor is whether or not he or she can reliably distinguish (1) all individuals or (2) marked cohorts in a population, or (3) neither. It does not matter whether the reptiles are naturally marked or marked by the researcher (see Chapter 9, “Marking Reptiles”). If all animals can be individually recognized, all of the techniques in this chapter are available. If neither individuals nor cohorts can be recognized, one can still address all abundance-state variables (range, occupancy, relative density, and absolute population density); however, the techniques suitable for absolute population density estimation are limited to small census plots, such as distance sampling and removal/depletion sampling with literal removal (see “Chapter Organization,” above). As I explain in much greater detail below (see “Absolute Estimators I”), removal/depletion sampling provides an estimate of detectability by gauging how fast the capture rate declines as animals are removed, either literally or figuratively. Figurative removal requires use of a cohort mark, i.e., animals receive a unique mark upon their first detection, so that the researcher will know whether a subsequent capture is new or a repeat. If cohort marks are not available, the alternative is literal removal (i.e., the reptile is killed, translocated, or kept in captivity until sampling is complete).

CLOSURE

Closure designates the condition in which animals neither leave nor enter a study population during the course of a study. The emphasis of closure is different with regard to occupancy from what it is with reference to density estimation. Because occupancy estimation establishes whether a given study unit is occupied, closure in occupancy models does not require that no individuals change places, but only that no unit ceases to be occupied or first becomes occupied during the course of a study. Thus, in a given occupancy study the number of lizards in a unit could change from 12 to 43 without violating closure, but it could not change from zero to one (unit becomes occupied) or from one to zero (unit ceases being occupied) without violating closure. In density studies, closure is violated if any individual appears or disappears from a study unit during the course of the study. Individuals may move around freely within the study unit as long as they do not cross the unit's perimeter.

Geographic closure occurs if animals neither enter nor leave the sampled area during the course of a study. *Demographic closure* occurs if animals are neither born nor die during the course of data collection. Closure is a very complex issue (discussed further in subsequent parts of this chapter), but is easy to appreciate how it could be important in some contexts. Consider a removal/depletion study in which 25 percent of the geckos living in a building are trapped each night. At first, 12 geckos are present, but this number declines by 25 percent through trapping each night. The second night about 9 are left, the third night about 7, and so forth. If the gecko population exhibits closure, the number of gecko captures will decline on a schedule such as 3, 2, 2, 1, 1, 1, 0, 1, 0, 0, 0, 1, 0, 0, 0 and 0. This exponentially declining capture rate will produce a precise and unbiased estimate of 12 geckos in the building. Suppose instead that closure is violated and that 2 geckos enter the building each night, taking over territories vacated by their captured comrades. If 25 percent of the geckos are captured every night, the number of captures will barely decline over time (e.g., 3, 3, 2, 2, 3, 2, 1, 3, 2 . . .); an analysis assuming closure would erroneously estimate that the building contains hundreds of geckos!

Closure is a key assumption for occupancy, removal/depletion, and some mark-recapture models. Indeed, the division I have used in this chapter between closed and open mark-recapture models highlights the central role of closure. In general, closed mark-recapture models are more precise and have powerful mechanisms for accommodating behavioral responses (e.g., trap happiness or trap shyness) and capture heterogeneity (unknown sources of variability in vulnerability to capture). However, if closure is violated, open and less-precise population models must be used.

Recall that density is defined as the number of animals (abundance) divided by the area occupied by the population. Closure violations complicate not only the estimation of abundance, for the reasons outlined above, but also the estimation of area. Imagine a situation in which 12 lizards occupy an isolated 0.5-ha rockslide but do not occur in the surrounding forest. In this case one can fairly easily ascertain that the density of lizards in the rock pile is 24/ha. However, consider a more realistic situation in which the rockslide is surrounded by smaller satellite rock piles located at a distance within the forest. At any given time a few of the resident lizards may be visiting one of the satellite rock piles. If the study site includes just the primary rock pile, one may still appropriately estimate abundance at 12 lizards, but confusion will exist regarding the area inhabited by those 12 lizards. Is it the area of the core rock pile, the area of the core plus satellite rock piles, or the area of the core plus satellite rock piles plus intervening forest? There is no agreed-upon approach to solving this common closure problem. Field biologists will no doubt be able to envision a host of additional examples in which the designation of places used intermittently by reptiles as inhabited or not is ambiguous. In other words, closure violations complicate and muddy both the abundance and area components of density estimation.

HETEROGENEITY

Any group that is composed of dissimilar elements is said to be *heterogeneous*. For example, a population may be heterogeneous (exhibit heterogeneity) with regard to survival if males typically die at a greater age than do females. Of greater import to investigators is heterogeneous detectability, which can pose a serious problem. Statistical inference is based on the assumption that individuals that are not sampled behave (at least in a statistical way) similarly to those that are sampled. However, individuals with low detectability are unlikely to be sampled because they are relatively hard to detect. Thus, the sample will be biased toward individuals that are easier to perceive, because those not sampled will have behaved differently.

The classical solution to this problem involves understanding the distribution of capture probabilities. Imagine a homogeneous population whose members on a given occasion can take on only two states: detected or not detected. This is exactly analogous to a coin flip; a resting coin can take on one of only two states: head or tail. The properties of flipped coins are very well understood, and the distribution of a finite number of flips can be rigorously modeled as a *binomial distribution*. A binomial distribution resembles a normal curve but is precisely shaped to reflect the constraint of having only two possible outcomes: head/tail (or detected/not detected). We are on statistically solid ground in assuming that the most likely outcome from 30 coin flips is 15 heads:15 tails; 10 heads:20 tails has a predictable but lower probability, and 29 heads:1 tail has an extremely low (but known) probability. Note that coin flips have a maximum likelihood of getting 50 percent heads and 50 percent tails, but it is a relatively trivial problem to generalize that outcome to any particular binomial proportion, say, a lopsided coin that produces 78 percent heads and 22 percent tails. This predictability in the shape of the binomial distribution is the phenomenon that allows statisticians to infer the distribution of detection probabilities when only part of the probability distribution has been sampled; that is, if we can see part of any binomial curve, we can estimate the rest of it. The more of it that we can see, the more precisely we can estimate the rest of it; with enough information we can infer its shape, central tendency, maximum likelihood, and so forth.

This is where heterogeneity comes in. If we are sampling coin flips from a population that is composed of two types of coins (one that produces 50 percent heads and another that produces, say, 43 percent heads), the resulting distribution of heads and tails will no longer be shaped like a binomial. If one fits a binomial to a nonbinomial distribution, one gets the wrong answer. The solution is simple: the two groups are analyzed separately, and each one is assigned its own appropriate binomial distribution. An investigator of population biology can deal with much of the heterogeneity encountered by identifying its source, grouping individuals according to the character states, and then analyzing the groups separately.

Latent heterogeneity is heterogeneity whose source cannot be identified and that cannot be associated with any identifiable factor. Latent heterogeneity is a problem especially when estimating abundance or density. Imagine a population that is composed of three groups of 100 individuals each. Members of the least-detectable group have a mean detection probability of 0.001; the other groups are composed of individuals with a mean detectability of 0.1 or 0.2. For ease of discussion we can call these groups juveniles, adult females, and adult males. The population is composed of 300 individuals, none of which is particularly easy to detect. With a reasonable number of detections, however, odds are that most would be adult males, a smaller number would be adult females, and perhaps a tiny sample would be juveniles. An investigator unaware of the heterogeneity in the population might try to fit a binomial curve to the number of captures. Because the bulk of the captures will be adult males, they will have the greatest influence on the fit of the binomial curve; juveniles, with the fewest captures will have almost no influence. Due to the disproportionate influence of the more easily caught individuals, the fitted curve will fall far to the right of (higher than) the true mean. Consequently, the investigator will overestimate mean detectability and, therefore, grossly underestimate true abundance.

This problem of latent heterogeneity affects estimates of occupancy, relative density, removal/depletion sampling, and most mark-recapture models. It can be the Achilles heel of almost any population estimate. If we knew that juveniles, males, and females had distinctly different detectabilities, we could carry out a new analysis based on three separate binomials, and the problem would be more or less solved. We would still need a large sample of juveniles for a reasonably precise estimate of total abundance, but at least we would be aware of the sample size requirements.

The benefits of understanding detectability and especially the basis of the heterogeneity in detectability evoke a general strategy for monitoring programs: identify detectability differences early on, so that sampling can be designed to maximize efficiency and minimize bias. Whether one is using simple capture rates or complex mark-recapture models, data collection will be less expensive and results more robust if the sources of detection heterogeneity are understood prior to data collection. Typically, most monitoring programs start off with insufficient funding and an inadequate understanding of the sampling problem. After years of counting, researchers begin to appreciate the complexity of a system and recognize that the data they have collected to date are biased to an unknown degree. Eventually, they start over after radically restructuring the data collection protocol; unfortunately, data collected in the first years of the study cannot be salvaged. The money wasted in the long run would have been better spent on understanding the system prior to data collection. A good way to start a new program is to investigate potential sources of variation and heterogeneity in detectability using a detectability-measuring method such as mark-recapture.

Point Estimates

In the remainder of the chapter I will examine single estimates of the three primary state variables: occupancy, relative density, and absolute population density. These are often called *point estimates* because they express the state of the system at a single point in space and time. A special subsection of the relative density section focuses on relative density estimation of commercially exploited species (see Chapter 16, "Studying and Monitoring Exploited Species"). In this case one is usually limited to obtaining yields or yield rates from commercial collectors, but the logic of this analysis can be used for any situation in which recoveries of dead individuals can provide insight into abundance. I have divided the discussion of absolute population density into six units: (1) small census plots, (2) removal/depletion sampling, (3) closed mark-recapture model, (4) open mark-recapture model, (5) combined open and closed mark-recapture models, and (6) estimating density from abundance and area. I conclude that section with strategic suggestions for obtaining the best point estimate of density. Once an investigator has obtained a sufficient string of density point samples, she or he can analyze them for trends over time, create life tables, and carry out population viability analyses.

Occupancy

The field of occupancy sampling has arisen over the last few years as a result of a series of brilliant publications by Darryl MacKenzie and colleagues (MacKenzie et al. 2002, 2003, 2005, 2006; MacKenzie and Bailey 2004; MacKenzie, Bailey, and Nichols 2004; MacKenzie and Nichols 2004; MacKenzie 2005a, 2005b, 2006; MacKenzie and Royle 2005; MacKenzie, Royle et al. 2004). Almost overnight, occupancy modeling has become the monitoring tool of choice for nationwide amphibian monitoring (Muths et al. 2006). While progress on this approach has been unsurpassed, it is still a little early to assess whether the approach will be as useful as it is hoped. The primary attraction is that the input data consist solely of species presence/absence data at easily defined sites. No handling, marking, or capturing is needed. An obvious advantage of this approach is the ease with which information can be obtained, implying that large-scale programs can be built around reports from volunteers. A less obvious, but potentially crucial, advantage is that because both occupied and unoccupied sites are sampled, one obtains information about the sites that do and do not support the species, which may facilitate decisions about the presence of detection heterogeneity. Most other methods ignore conditions associated with failure to detect.

The basic procedure in occupancy sampling is to visit each site several times during a season of interest, noting whether a species is present or absent. These data are entered into a user-friendly free software package, PRESENCE (MacKenzie and Hines 2002–2010), for processing; the comprehensive population-biology program MARK (Cooch and White 2005), a free download, can also be used. The analysis provides average detection probabilities for the species and an estimate of occupancy along with associated confidence intervals. Program PRESENCE may judge, for example, that 54 percent of the sites visited most likely were occupied by the species of interest even though it may have been found at only 43 percent of the sites (which is termed the *naive occupancy estimate*). It is the incorporation of detectability into the analysis that leads to the difference between the naive estimate and the estimated occupancy. Program PRESENCE estimates detectability on the basis of the frequency with which the species was detected, assuming that if it was detected by any observer during any visit to a site, it was present at that site throughout. Suppose, for example, that the site was visited four times by a pair of observers who recorded their observations separately. Suppose further that the species was detected at three of the eight possible times. From that single site example, PRESENCE would judge the detectability to be 3/8, or 0.375. For the simple analysis described, however, PRESENCE makes the further simplifying assumption that detectability is the same at all sites; thus, it draws from all of the site visits to estimate mean detectability. Occupancy is projected from the number of actual detections, each adjusted for the likelihood that a resident population was overlooked due to incomplete detectability.

This is a powerful analysis, made easier by the ability of PRESENCE to accommodate missing data (the number of visits need not be the same for all sites) and group and individual covariates. An example of a group covariate might be habitat; if detectability varies by habitat, one could assign a habitat code for each site such that detectability or occupancy was a function of habitat. An example of an individual covariate might be rainfall. If rainfall influences detectability (or occupancy), PRESENCE can model this continuous function if the amount of rainfall is recorded for each visit and entered in PRESENCE as a covariate tied to each visit.

The analytical model we have been discussing is the one for single-species, single-season occupancy. The five key assumptions for this analysis (MacKenzie et al. 2006) are as follows:

1. The occupancy state of the sites does not change during the period of the survey (closure).
2. The probability of occupancy is equal across all sites (geographic homogeneity in occupancy).
3. The probability of detecting the species (if present) in a survey is equal across all sites (geographic homogeneity in detectability).
4. The detection of the species in each survey of a site is independent of detections during other surveys of the site (temporal independence of detectability).
5. The detection histories observed at each location are independent (geographic independence of detectability).

Keep in mind that “is equal across all sites” allows for random error; that is, the scores may be scattered around the same mean value. They need not have exactly the same value and be measured without error, but they do need to be similar enough to have been drawn from the same underlying homogeneous distribution.

The plausibility of these assumptions needs to be evaluated case by case, but one can imagine a variety of circumstances in which they would be invalid. For example, consider the closure assumption. Recall that the investigator has complete freedom to adjust the size of the sites being searched during a study (size of sites should not vary among sites within a study). If exceptionally small sites are chosen, a large number of sites will be occupied by just a single reptile individual, in which case closure is likely to be routinely and frequently violated. In contrast, if sites are large relative to resident population size, closure violations are apt to be few, but occupancy might be so high (i.e., essentially all sites are occupied) that occupancy measures would be uninformative as a monitoring tool. A useful rule of thumb suggested by MacKenzie and colleagues (2006) is that occupancy monitoring is useful only if occupancy rate is between 0.2 and 0.8.

Occupancy analysis in herpetology is most commonly applied to pond-breeding frogs. In North America, many of these frogs deposit large clutches, such that an occupied pond is likely to have thousands of eggs or tadpoles, whereas an unoccupied pond will have none. Single-season closure is unlikely to be violated in such a situation. With reptiles, however, one is much more likely to encounter sites in which just a single individual is present, with attendant high risk of closure violation. Another problematic situation with reptiles is the seasonal appearance of young-of-the-year (YOY). Naive young lizards, in particular, tend to occupy sites that were not occupied prior to their emergence and will not be occupied later in the year when mortality thins the numbers of the YOY cohort. One solution to this problem is to limit occupancy sampling to the pre-emergence season, or to adults only.

The assumption of geographic homogeneity in occupancy also seems more likely to be satisfied with pond-breeding frogs than with most reptiles. As with most other forms of heterogeneity, it can be addressed by stratifying one’s sample by features known to create heterogeneity (e.g., habitat type). Nevertheless, I believe that a wide variety of circumstances exist in which latent heterogeneity in occupancy would occur for reptiles. With sites of sufficient size, such heterogeneity may disappear, but then one is likely to exceed the 80 percent occupancy ceiling for useful application of occupancy models. Applying occupancy sampling to small sites runs an appreciable risk of revealing unrecognized habitat or microhabitat heterogeneity and thereby violating the assumption of geographic homogeneity in occupancy.

The assumption of geographic homogeneity of detectability is an assumption with special challenges, because the number of individuals within an occupied site can vary from one to hundreds or thousands. Consider the problem of detecting a single individual at a site. As someone charged with detecting incipient populations of invasive snakes, I can assure you that finding a single reptile individual can be very difficult (i.e., the detection probability for a single snake can be exceedingly low). However, the probability increases sharply with an increase in the number of snakes. It roughly doubles with just two individuals, for example. As the number increases further the increment becomes proportionately smaller, but the full range of possible change is quite high. Detection probability for a large population can be very high, even approaching 1.0. The correlation of detectability with local abundance is of great concern to occupancy researchers (see MacKenzie et al. 2006, chapter 5) and is likely to be pervasive in reptile monitoring programs. To date this has no obvious solution, although MacKenzie and colleagues (2006) have encouraged readers to take note of some new count-based models being developed by Andrew Royle (see “Relative Density,” below).

The assumption of temporal independence of detectability is most readily violated by double-observer data collection, in which two or more observers collect data during each visit to a site. If some temporal phenomenon, such as weather, affects all observers equally, their records will not exhibit temporal independence of detectability. If weather-related causes of variation in detectability can be adequately measured with covariates (e.g., the amount of rain falling at the site in the 6 hours preceding a visit), this temporal variation can be accommodated without violating the fourth assumption. In my sampling of reptiles, however, I have routinely observed statistically large temporal effects that I have been unable to tie to measured weather covariates.

The final assumption—geographic independence of detectability—is most easily violated if sampling units are very small. For example, if two sites are near enough to each other that individuals shift back and forth, detectability might not be geographically independent. In general, however, this assumption is unlikely to create major difficulties and can often be resolved with a well-designed sampling protocol.

The single-season single-species model described above has been generalized in a number of useful ways to estimate multiple-season occupancy, multiple-species occupancy, interactions among species, and even community level analyses (MacKenzie et al. 2006). MacKenzie and colleagues also provided a number of useful guidelines for the design of occupancy studies. With regard to the size of the site sampled, they urged investigators to consider management practices (e.g., the optimal size for a management unit) and to recognize that smaller units have lower mean occupancies. The larger the area sampled, the more likely it is to contain at least one individual of the focal species. By adjusting the size of the sampling unit, one can ensure that the mean probability of unit occupancy falls in the preferred range of 0.2 to 0.8. MacKenzie and colleagues (2006) also urged researchers to recognize the trade-off between larger sample size (longer sampling season) and temporal heterogeneity (likely to increase with longer sampling seasons), keeping in mind that management objectives may be better met by particular time intervals.

The optimal number of visits per site (assuming that the cost of each visit is equal) is summarized in Table 28. MacKenzie and colleagues (2006, chapter 6) illustrated procedures for modifying the equal-cost assumption to suit a specific situation. They noted that the number of repeat surveys is optimal when the probability of a false absence falls in the range of 0.05 to 0.15. The probability of a false absence can be estimated as

$$p_{\mathrm{fa}} = (1 - \text{detectability})^K \qquad (1)$$

where K is the number of visits. Thus if the chance of detecting a species on a given visit is 10 percent (detectability = 0.1), then the probability of failing to detect it on a given visit is 0.9 (i.e., 1 − detectability). To decrease the probability of a false absence, one needs to increase K. To solve for K, in the equality $0.15 = (0.9)^K$, take logs of both sides of the equality

$$\log(0.15) = K^{*}\log(0.9) \qquad (2)$$

or rearranging,

$$K = \log(0.15)/\log(0.9) = 18.006. \qquad (3)$$

Thus for this circumstance, decreasing the probability of a false absence to 0.15, 0.9 needs to be raised to about the 18th power, so the optimal number of visits per site is 18 or more. MacKenzie and colleagues (2006) recommended that if detection probability is likely to be heterogeneous, investigators should err on the side of additional visits. A particularly favorable situation occurs if detectability is high (~0.5) and occupancy is mid-range (~0.5), in which case 3 is the optimal number of visits (Table 28). This underscores the efficiency of occupancy sampling when detectability is high (i.e., >0.3).

Under certain circumstances one can further improve the efficiency of occupancy modeling by using a removal sampling design in which visits to a site stop as soon as the species is recorded there (Azuma et al. 1990). Additional visits can only confirm that the species is still there, so it is more profitable to shift sampling effort to sites where occupancy has not been determined. MacKenzie and colleagues (2006, p.180) stated that "a removal design is likely to be more efficient than a standard design if detection probability is relatively constant, but may provide less flexibility in modeling."

Because occupancy sampling is relatively new, it is too early to determine its utility for monitoring the health of large scale populations. Occupancy sampling is an easy and inexpensive tool, and it should be especially effective in sites where colonization and extinction are relatively frequent. It is unlikely to be as effective near the center of a species' range, at sites where occupancy is near 100 percent, or with species of low detect-

TABLE 28

Optimal Number of Surveys to Conduct at Each Site When All Sites Are Surveyed an Equal Number of Times and All Surveys Cost the Same[a]

Detectability	*Occupancy*								
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0.1	14	15	16	17	18	20	23	26	34
0.2	7	7	8	8	9	10	11	13	16
0.3	5	5	5	5	6	6	7	8	10
0.4	3	4	4	4	4	5	5	6	7
0.5	3	3	3	3	3	3	4	4	5
0.6	2	2	2	2	3	3	3	3	4
0.7	2	2	2	2	2	2	2	3	3
0.8	2	2	2	2	2	2	2	2	2
0.9	2	2	2	2	2	2	2	2	2

[a] From MacKenzie and Royle 2005.

ability (<0.2). Because occupancy modeling seems highly vulnerable to latent heterogeneity in detectability, I doubt its precision and accuracy for most reptiles. It may be too imprecise to be of much value as an index of overall species abundance, but it seems the obvious choice when occupancy is the stated variable of direct interest.

Ultra-Rarity

Program PRESENCE (MacKenzie and Hines 2002–2010) was designed to quantify the average occupancy rate and works best for occupancy rates in the 0.2 to 0.8 range. In some circumstances, however, the element of interest may not be averages but rather the presence/absence of a single individual or a single tiny population. One may wish to confirm the presence of a potentially invasive species, or a nature reserve manager may need to know, for regulatory reasons, whether a species near the edge of its range occurs in the reserve. The basic approach in such instances is the same: estimate the detectability of the species and survey the study area enough times to reduce the probability of false absence to whatever statistical level is desired (Reed 1996). Recall that the probability of a false absence, $p_{\text{fa}} = (1 - \text{detectability})^K$, where K is the number of passes needed to produce the requisite probability. Recall also that if the manager wants a 95 percent assurance that the species is not present, the probability of false absence should be <0.05.

This simple estimation procedure assumes that searches are independent and the probability of detecting a single individual of the species (p) is known. Mark-recapture and removal/depletion studies provide good estimates of p (both occupancy and mark-recapture models use the symbol p to represent detectability, but occupancy p represents the detectability of a population, whereas mark-recapture p represents detectability of an individual). Unfortunately, estimating individual detectability at a site where a species is rare or possibly absent is often impractical. Instead, one estimates p at a site where the species is more common and assumes that individual detectability is unchanged at lower densities. Is that a realistic assumption? Too few data have been collected on this issue to extract general conclusions, but one can envision scenarios in which it may not be valid. If the target species has a social component to its behavior, p may depend on population density (Reed 1996). For example, a turtle may bask or enter a basking trap more readily if other turtles are already doing so (when neighbors are few, the likelihood of trap entry declines precipitously). Such "social facilitation" causes detectability to be sensitive to population density. Likewise, the Brown Treesnake (*Boiga irregularis*), in areas on Guam where is common, depletes its prey, which on average causes it to be hungrier, which facilitates its entry into food-baited traps (Rodda et al. 2002; Boyarski et al. 2008). Thus, detectability of individual Brown Treesnakes (using food-baited traps) is much higher (about seven-fold) where they are common than where they are rare (Gragg et al. 2007). Investigators must exercise great care in extrapolating individual detection probabilities from areas of normal densities to situations of ultra-rarity.

Relative Density

An index of density is the preferred method of quantifying population density when more rigorous methods are prohibitively difficult or expensive. Naturally, the first and best step is to learn as much as possible about the ecology, behavior, and detectability of a study species. If the species is often found in light gaps, the sampling protocol should quantify light gap availability. If the species is rarely seen on days when the temperature drops below 14°C, then temperature must be recorded often and in places germane to the microenvironments occupied. If ecological/behavioral data on the study species are few, one should consider generalizing from better-studied species that are closely related or have similar habits.

Three broad categories of factors typically affect the detectability of a species: species attributes, observer biases, and environmental context (see Table 14, in Chapter 12). Species attributes that influence detectability include the size, sex, maturation, reproductive condition, satiety, and parasite loads of the target individuals. Environmental context includes weather conditions, season, habitat, microhabitat, sighting distances, and type of trap attractant. Important habitat features depend on the biology of the study organism and can include such seemingly esoteric features as availability of water-holding tree holes, number of rocks with cavities underneath, sun exposure of sandy slopes during the nesting season, or the extent (m^2) of tree trunks with loose bark. Vegetation maps can be helpful for quantifying habitat, but more subtle features can also affect detectability. For example, the detection fraction of a terrestrial lizard that hides under coarse and fine woody debris might depend on the relative amounts of each (see Hyde and Simons 2001).

Sampling effort is another important factor and must incorporate some measure or estimate of the relative detection success of different observers (Sugerman and Hacker 1980; Rodda 1993). Observer skill probably interacts statistically with other factors, such as weather, that may affect detection rate, so all combinations of conditions should be recorded. One observer, for example, may be better than other observers at spotting small animals, and small animals may extend their activity seasons longer in the fall than larger ones. In such cases, searcher(s), weather, and animal size should be recorded along with sampling results. Some sampling designs allow an investigator to pool observations in a way that will balance or average out the influence of varying sighting conditions (Thompson et al. 1998), but the opportunity to pool data during analysis provides a decisive solution only if the requisite data were collected and are understood.

When zero individuals of a target species are taken at a given study site in a particular year, it may be tempting to stop surveying the "unproductive" site. Investigators should resist the temptation and cherish the value of their negative data. The site may be occupied by the species the following year, or perhaps the search was conducted under inappropriate weather conditions. Data that demonstrate which type(s) of weather is "wrong" may be exceedingly valuable for scheduling future surveys. Furthermore, data showing the absence of a species may provide insight into the conditions necessary for the species to thrive.

The analysis of relative density estimators follows conventional statistical practice, with no special software or programming for comparing groups, analyzing trends, and so forth. One potential alternate approach is the new method developed by Andrew Royle to infer absolute population densities from simple counts (Royle and Nichols 2003; Royle 2004a, 2004b). The method assumes a specific mathematical distribution for count data and computes absolute population density based on the fit of the counts to the presumed underlying distribution. A comparison of results from Royle's method with those from a conventional closed-population mark-recapture study (Besnard et al. 2007) showed that Royle's population estimate was about 25 percent of the mark-recapture estimate. Validation studies of mark-recapture show that mark-recapture tends to underestimate abundance due to latent heterogeneity (Rose and Armentrout 1974; Boulanger and Krebs 1994; Williams et al. 2002; see also "Hard Validation," under "Validation of Techniques and Assumptions," in Chapter 12); thus, finding a grossly lower value is not very affirming for Royle's method. As noted above, I am skeptical of the validity of this approach for most reptile species, but much additional experience with it is needed before it can be adequately evaluated.

Absolute Estimators I: Small-Plot Censusing, Distance Sampling, and Territory Mapping

Although it is rarely practical to census (as opposed to survey) a study population, censusing small representative plots within a study population and then estimating demographic characteristics of the study population on the basis of the sample censuses is often feasible. In this section we will consider three types of small plot sampling. In small-plot or quadrat censusing (quadrat sampling), the area sampled is small enough to be inspected so thoroughly that all individuals of the focal species are detected. In distance sampling, which is carried out on either linear transects or circular point networks (trapping webs), animals on the lines or at the points are censused, whereas those farther away are merely surveyed. Finally, in territory mapping, a census technique for visually conspicuous animals that behaviorally exclude conspecifics from their areas, an observer obtains a census by mapping the boundaries of each territory.

SMALL CENSUS PLOTS

Quadrat sampling is discussed in Chapter 13 (see "Quadrat Sampling"). One type of quadrat is the "*litter plot,*" which has a long history in herpetology (Brown and Alcala 1961, 1964; Sexton et al. 1964); more recent variations include micro (1-m^2) litter plots (Rocha et al. 2000) and total removal plots (Rodda, Campbell, and Fritts 2001). Ordinary statistical practices are used to extrapolate from a sample (of plots) to a population. The unique challenges associated with small census plots stem from their small size and, therefore, relatively long perimeter, which provides numerous opportunities for violation of closure through animal movement. Heatwole (in "Quadrat Sampling," Chapter 13) amply demonstrates the hazards of closure violation. Because the plots are small, the extrapolation of the sample will be substantial; therefore, plots must be carefully placed to ensure representativeness of the sample. Small size allows the plots to be placed virtually at will, but Rodda and colleagues (Rodda, Perry, et al. 2001) reported a situation in which a 1-m^2 sampling frame gave misleading results, because the rigid sampling frame could not be inserted through the trunks of trees. Apparently, a small lizard that preferentially seeks refuges at the base of trees was grossly undercounted because the sampling had to be placed away from tree trunks.

DISTANCE SAMPLING

Distance sampling (see "Transect Surveys, Including Line Distance]," Chapter 13) is a special type of transect sampling in which the observer records the perpendicular distance from the transect centerline to each detected animal. Using the distribution of these detection distances, program DISTANCE (Buckland et al. 2001; Thomas et al. 2010; www.ruwpa.st-and.ac.uk/distance/) estimates the relative detection function (i.e., the relative proportion of the animals that are detectable at each distance from the centerline). If one assumes that the peak detection probability is 1.0 for the individuals in the small strip immediately adjacent to the center line, then those individuals have been censused, not just surveyed. If the density of individuals in that small strip is characteristic of the entire population, then the population's mean density can be estimated from the local density observed near the centerline. The key assumptions are that (1) all individuals near the centerline are detected, (2) the animals do not approach or flee from the observer before being detected, (3) the centerline is placed randomly in relation to animal density (i.e., not on a trail or road), and (4) that distance measurements are without error. If these assumptions are correct, one can plausibly estimate average absolute population density of the study area from the small strips or plots actually sampled. The assumptions and applicability of distance sampling are explained in Chapter 13 (see "Transect Surveys, Including Line Distance"); they have been reasonably well documented (Burnham et al. 1980; Buckland et al. 2001) and will not be further examined here.

Trapping-web sampling is a radial variant of linear-transect distance sampling, in which traps are set in a hub-and-spoke arrangement. The density of traps at the hub must be high enough to census individuals near the hub. At increasing distances from the hub, capture success declines in a manner exactly analogous to the drop-off in capture success with distance from a transect centerline. The essential datum for each trapped individual is the distance from the hub's center to the point of capture.

DATA ENTRY

For both linear transects and trapping webs, the recorded distances are input into program DISTANCE, which will estimate the proportion of the animals detectable at each distance from the centerline. The investigator can enter each distance measurement individually, or she or he can group distances into bands and enter the number of captures within each band. Those are all the data needed to run DISTANCE.

The results (counts and transect lengths) from all passes of any transect that is searched repeatedly should be pooled (summed) and entered as a single sample to avoid pseudoreplication, or the double counting of samples that may not be independent (see "Pseudoreplication," under "Precautions for Quantitative Reptile Field Studies," in Chapter 3). To constitute different samples, distances from the centerline to individual animals along transects must be independent. Consider a transect with certain highly favored perches that are frequently occupied. The distances from the centerline to those favored perches would likely appear each time the transect was searched, and those distances would be pseudoreplicated.

One expects the number of detections to decline at increasing distances from a centerline (or hub), but it is difficult to anticipate a more specific function than a simple monotonic (i.e., no reversals) decrease. For example, if the species is uniformly distributed and all of the animals in the innermost three bands are detected, one would expect a flat sighting distribution function out to the fourth cut point, but a decline thereafter. Conversely, it may be that only the innermost band provides 100 percent detection, but the decline with increasing distance is very shallow, with nearly 90 percent being detected in the outermost band sampled. Because there is little or no theoretical basis for expecting a particular slope or shape of the detection curve, DISTANCE can attempt to fit almost every possible shape and slope. The basic shape is described as a *key,* with modifications termed *adjustments*. These should be reported in publications for the purposes of replication by other workers, but they are not in themselves of any special interest. The curve fit is needed only to estimate the y intercept and its confidence limits (the rest of the curve is ignored, except for what it suggests about the y intercept). For well-behaved data (i.e., assumptions met, no violation of the assumption of monotonic decrease at greater distance from the centerline), program DISTANCE will do an excellent job of estimating the population density and will be relatively insensitive to the choice of key and adjustment. If the data are poorly behaved, one should investigate the legitimacy of distance sampling for the system. DISTANCE has goodness-of-fit tests that will help measure the match between the distance histogram and the chosen key/adjustment.

HEAPING

When distances are estimated rather than measured, certain round numbers tend to be overrepresented in the sample. For example, "10 m" is likely to show up more often than 9.7 m. This tendency is called *heaping*. Heaping can produce artificial zigs and zags in the distance histogram (i.e., the function will not be monotonic), and heaping can bias the estimation of density. Suppose, for example, that most distances are recorded to the nearest meter and that cut points for the distance histogram are then chosen to be 0.1, 1.1, 2.1, 3.1 . . . meters. Choosing such cut points eliminates heaping, but because the mean distances for the different intervals are 0.6, 1.6, 2.6, etc., meters and the bulk of the corresponding measurements would be 1.0, 2.0, 3.0 . . . meters, some distance bias will occur as a result of measurement approximation. It is best to measure the distances reasonably accurately, although unbiased error in distance estimation does not appreciably distort population estimates based on line transects (it is more critical for radial measurements from trapping webs).

TRUNCATION

Truncation is the process of shrinking a value or sample to a specified threshold; in the context of distance sampling, truncation shrinks the sample by eliminating the records farthest from the observer. Inclusion of outlier distances in a distance analysis can distort estimates, just as outliers near the corners of a regression plot exert an undue influence on the slope of the fitted curve. Truncation of the data can reduce bias in a density estimate and improve precision of the point estimate by emphasizing the crucial nearby distances. Buckland and colleagues (2001) suggested that the outer 5 percent of distance measurements be dropped. A more precise method is to perform a preliminary fit of the curve and truncate the data set beyond the point at which the detection fraction is estimated to be 5 to10 percent of the maximum. Due to limited visibility, problematic outliers occur only rarely in surveys of most terrestrial reptiles, although outliers may distort aerial surveys of marine reptiles.

APPLICABILITY

Distance sampling is most appropriate for sampling conspicuous species that are restricted to a two-dimensional plane that does not include the observer. For example, active desert tortoises are almost always found in the two-dimensional plane of the desert floor. A walking observer can sometimes look down onto the plane of the tortoise, without having to look through the shrubs that dot the plane. Dense vegetation is unfavorable for distance sampling by sight (Rodda and Campbell 2002). Sea turtles swimming on the surface are among the best candidates for distance sampling from the air.

Unfortunately for our purposes, sea turtles dive and desert tortoises burrow. Therefore, DISTANCE estimates the instantaneous density of individuals on the surface. Because submerged or subterranean animals are not part of the study population, an essential component of estimating the size of the entire population is determining the fraction of the population that is below the surface at any given moment. One could hope that the detection fraction is constant, but realistically it is likely to vary with temperature, wind speed, time of day, season, and so forth. Typically, these environmental conditions and the animal's location vis-à-vis the surface are monitored with radio telemetry and analyzed with conventional statistical software to construct a logistic regression model of surface activity. Total population density is then computed as the product of the estimated surface density and the inverse of the estimated proportion of the population on the surface.

One problem with taking measurements and then applying multiplicative corrections to them is that confidence limits tend to balloon with the inclusion of additional parameters. In order to estimate population density, for example, one might need to estimate not only the relationship between detectability and distance from the centerline but also the role of wind speed and season in modulating reptile activity. DISTANCE software and many statistics books provide instructions for estimating confidence limits of parameters that are multiplied together. Required inputs are the best estimators for each parameter, their confidence limits, and the covariances among the parameter estimates.

TERRITORY MAPPING

Highly visual and territorial animals can sometimes be censused by mapping their territorial displays. Typically, adults patrol the perimeter of their territories, displaying at regular intervals. Same sex conspecifics are chased away, but males typically encourage a number of females to reside in their territories. If the study species is such an animal, and if its territorial interactions can be viewed without the observer's presence distorting the shapes of the territories, the territories can be mapped to enumerate the number of territorial adults present. Mapping would not be necessary if all individuals were visible simultaneously, but vegetation or substrates are likely to conceal some individuals at any given sample. Nonterritorial adults and juveniles tend not to be counted with territory mapping.

An advantage of territory mapping is that disturbance of the monitored individuals is minimal. The animals need not be captured, handled, or marked. If marking is omitted, however, the investigator will need a mechanism for recognizing the animals individually to avoid double counting or undercounting. Well-studied individuals can often be recognized individually, but such recognition may be labor intensive. If the population exhibits a predictable spatial arrangement (e.g., male territories never overlap), one may not have to study each territory in detail, as a count of the territory holders in a saturated area will establish the population density, even without information on the location of a given boundary.

Determining the number of territory holders is analogous to removal/depletion sampling (see "Absolute Estimators II," below). In each process, the number of unrecognized/unmarked animals should decline exponentially with constant effort. Thus, one can use the analytical tools mentioned in the Absolute Estimators II section to extrapolate a reasonable estimate of the total population size from a data accumulation curve that includes most territories.

One limitation associated with reliance on territory mapping as a population-monitoring device is that a population can decline substantially without a concomitant decline in the number of territories. Up to a certain—usually unknown—point, territorial vacancies in a declining population are filled by previously nonterritorial adults. Thus, initial population declines are manifest in sharp depletion of the number of nonterritorial adults (typically not surveyed), but little or no change in the number or density of territories. Similarly, population increases beyond a territorial saturation threshold may be difficult to detect with territory mapping.

Absolute Estimators II: Removal/Depletion Sampling

If one were to capture and hold half of the animals in a closed population one day, and if the probability of capture were invariant among days, then the number of captures the next day should be only half the number on day 1, as only half as many animals would be available for capture. This idea has been formally developed into an approach called *depletion* or *removal sampling,* in which a curve is fitted to the slope of the decline in new captures to estimate capture probability. A steep decline in captures implies a high capture probability, whereas a shallow decline in number of captures indicates proportionately poorer capture success (or closure failure). Because removal sampling is a name that has also been used to describe intensive sampling in which both vegetation and animals are removed (Rodda, Campbell, and Fritts 2001), referring to *capture-rate-decline analysis* as depletion sampling may prevent confusion. Using the known number of captures and the rate of population depletion, one can infer the size of the population being depleted.

It is not necessary to deplete the population literally; one can figuratively deplete the population by mark-release, counting only previously unmarked animals. This is sometimes called *removal by marking.* Removal by marking depletes only the population of animals that are unmarked. Indeed, it is often biologically advantageous to deplete figuratively rather than literally. For territorial animals, which are most likely to satisfy the closure requirement of depletion sampling, literal removal of territory holders is likely to induce social disruption, as animals lacking territories take over the spaces vacated by animals that are removed. Territory takeover violates the closure assumption if the new territory holders immigrate into the population. Figurative removal by marking, which is much less likely to induce social disruption and movement, consists of repeated sampling occasions, each one of which consists of inspecting each captive for marks and adding marks if necessary. Because the input data for depletion sampling are exactly the same as those with closed models, and closure is required for both, mathematically, depletion analysis is a special form of closed-model mark-recapture analysis. The handling of removal/depletion data are explained below (see "Closed-Model Mark-Recapture").

ASSUMPTIONS AND LIMITATIONS

Closure is the most problematic assumption for depletion sampling, which is often done quickly (over a few days) to minimize opportunities for immigration. Closure violations typically lead to overestimation of true population size, because of animals entering the plot during sampling. Flight from the sample plot will cause underestimation, although overestimation seems to be the more frequent problem, and it can be severe. Heterogeneity of capture probability can be a problem, as depletion is rarely sustained long enough for detection and quantification of the more refractory individuals. Marks, of course, must not be lost, misread, or misrecorded. In practice, a fairly high capture probability is desirable so that a usable set of capture histories can be obtained before the violation of closure becomes consequential. The combined need for high capture probability and absolute closure is difficult to meet for most reptiles, although some species are well suited to this method. White and colleagues (1982, p. 101) commented, "If all animals have an average capture probability of at least 0.8, two [sampling] occasions will suffice because failure of constant capture probability will not matter . . . To obtain useful results with the removal method, capture probabilities should be at least 0.2, and if they are as low as 0.2, even six capture occasions will give only marginally reliable results."

Shortening the time interval over which depletion sampling is conducted will reduce the opportunities for closure failure. At the extreme, this can create a different type of bias: chronic flight or concealment from the searchers. Consider a situation in which a group of investigators intensively searches the vegetation in a 1-ha plot for 1 h. In our example, the species being sought takes refuge underground. At the conclusion of the first search hour, the searchers start over, searching the same plot for another hour. Likewise, the search is repeated for a third hour. It is likely safe to assume that no animals are born or die in 3 hours. The number of animals captured in each hour will probably decline over the three searches, and the rate of decline has been used to estimate total population size (e.g., Tanaka et al. 1999).

Two problems can arise in such a scenario. First, if a significant fraction of the population is inactive and inaccessible on a particular night, then it will not be part of the sampled population even though it is part of the population of interest. For example, snakes that have eaten a recent meal or are about to shed or reproduce may remain underground and, therefore, be undetectable. Second, if an active snake can conceal itself in response to the approach of a human searcher, it can effectively disappear (stay hidden) from the detectable population for all subsequent sampling hours. Both of these problems are elements of geographic closure (underground individuals are outside the study area, i.e., the surface of the ground). This can be largely alleviated by spreading the surveys over time intervals long enough for the target individuals to leave their refugia and again become detectable; doing so, however, increases the risk of conventional closure being violated (animals wandering out of or into the geographic area, or dying or being born). The optimal balance depends on the biology of the study species.

SOFTWARE

Removal or depletion sampling bears many similarities to standard mark-recapture, with the special condition that recapture probability is, by definition, zero. This special circumstance is accommodated by most mark-recapture software packages. The most widely used are MARK (Cooch and White 2005) and CAPTURE (White et al. 1978; Rexstad and Burnham 1991; http://www.mbr-pwrc.usgs.gov/software/capture.html). Data entry and formatting are the same as for ordinary mark-recapture, although the data type option in the program will need to specify zero recapture probability.

Absolute Estimators III: Closed-Model Mark-Recapture

Closed-model mark-recapture is the method of choice for estimating population density and abundance. If the assumptions of the model are met, it is the most precise mark-recapture model. Because birth, death, emigration, and immigration are believed to be nonexistent in a closed population, those parameters do not need to be estimated if the population is truly closed. Because parameters interact mathematically in a population model, any increase in the number of parameter estimates increases uncertainty; thus minimizing the number of applicable parameters in a model improves precision. Closed models are conceptually fairly straightforward, with excellent and well-documented software for data analysis, in particular, the program CAPTURE (Appendix II) and its associated publications (White et al. 1982; Cooch and White 2005).

MODEL NOTATION

Otis and colleagues (1978) introduced a notation system for mark-recapture models that has been widely adopted. The notation focuses on the assumptions on which the models are based. M_0 designates the analytical model for which all key assumptions are satisfied, that is, the zero or null model for which all assumptions must be met for the model to provide unbiased analysis. The M_b model allows the behavior assumption—that animals do not change their behavior in response to capture—to be violated. Recall that statistically, "capture" includes any form of contact with the animal, including sighting, remote marking, or even detection from DNA samples. Trap captures are somewhat special in that an animals can learn from the experience to recognize that a unique device placed in a particular place in their activity range means capture. Each animal can henceforth avoid (trap shyness) or seek out (trap happiness) the device. If no other mark-recapture assumptions are violated, the M_b analytical tool is appropriate. M_t is the time model, which allows capture rates to vary with time (e.g., the capture rate on day 2 is double that of day 1), but the other assumptions must be met. Finally, there is M_h, which allows for individual heterogeneity in capture probabilities (e.g., some individuals are hard to catch; others are easy), but the other assumptions must be met. Other analytical models combine these features. Model M_{tb}, for example, simultaneously accommodates a behavioral response and capture variation over time. The power of the analysis declines as additional nuisance parameters are incorporated. With current analytical software (e.g., program MARK, which includes program CAPTURE), all eight permutations can be analyzed.

Some of the models have additional names that are closely associated with their originators or their mathematical method. Thus, M_h may be called the Jackknife Estimator; $M_{t,}$ the Darroch Model; and $M_{b,}$ the Zippin Model or estimator. In addition to the assumptions indicated by the above subscripts (t, b, h), there are three assumptions for which extant closed models have no flexibility: (1) the population is closed, (2) animals do not lose their marks, and (3) all marks are correctly noted and recorded on each sampling occasion. Later in this chapter (see "Open-Model Mark-Recapture," below), I will discuss open models, under which assumption (1) can be violated. Program CAPTURE provides a test of closure, but the test rarely rejects the null hypothesis of "no closure violation" unless the data set is large and the violations are flagrant. Many reptile data sets will pass the CAPTURE closure test whether they are closed or not.

White and colleagues (1982) created CAPTURE to support analyses explained in their landmark monograph (Otis et al. 1978; also see White et al. 1982). CAPTURE is still used, and a Windows-based version is available with program MARK, as noted above. CAPTURE is novel in providing a subprogram that endeavors to identify the most appropriate analytical model ($M_{b,}$ M_{tb}, etc.) to use when analyzing a given data set. Subsequent study has shown, however, that the model selection algorithm is not very powerful and often chooses an analytical model that is suboptimal, particularly if the data are sparse (Menkins and Anderson 1988).

MODEL INPUT

Input for a closed model consists of a string of zeros and ones that correspond to the detection history of each known individual in the population. Suppose, for example, that animal X was seen on the first sampling occasion, not seen on the second and third occasions, and resighted on the fourth occasion. Its capture history would be 1001. Suppose further that one is using analytical model M_0, which assumes that all individuals have the same capture probability, which is constant over time and which does not change in response to capture experience. For simplicity, let us further suppose that mean capture probability in this particular case is 0.2, indicating that on an average occasion, 20 percent of the population will be detected. Therefore, an animal with a capture history of 1001 would have an overall capture probability of $0.2 \times 0.8 \times 0.8 \times 0.2$, reflecting that the animal was captured on the first occasion (with a probability of 0.2), was not captured on the second and third occasions (probability of not being captured on a given occasion is $1 - 0.2$, or 0.8), and so forth. The product of these probabilities, 0.0256, is the probability that the capture history 1001 will appear in a large sample of capture histories if the M_0 model is correct and capture probability averages 0.2. For a given analytical model and set of capture histories, the computer "tries" various capture probabilities to determine which most closely corresponds to the array of capture histories that has been observed. The computer does this for each analytical model in turn and then compares the goodness of fit of the observed data set to the distribution expected under each of the conceptual models. The preferred model is usually the one with the best fit (see "Assessing Relative Model Fit" and "Assessing Absolute Model Fit," below).

Suppose in this case that M_b fits the data best and estimates a population size of 117 animals (95% confidence limit [CL] 83–148). The fact that a model incorporating a behavioral response (M_b) provides the best fit suggests that the animals have become either trap shy or trap happy (inspection of the program output will indicate which). That information and the estimated population size may be useful, as is the finding that other models (M_t, M_h, etc.) do not describe the observed array of capture histories as well. This does not mean that there is no temporal change or individual heterogeneity in capture success, but rather that the amounts of temporal change and individual heterogeneity are insufficient to justify incorporation of those parameters into the analysis of the given data set. With additional information (e.g., a larger data set), inclusion of additional parameters might be justified.

DEFINITIONS

What is an *occasion*? Consider a grid of traps that has been set overnight. On the first morning, three animals are found in traps. Those individuals are said to have been detected on occasion 1. The following day the traps are checked again, constituting occasion 2. Now consider a recapture effort spread over an area so large that it cannot be completed in a single day. Suppose that one is detecting turtles visually, in a series of ponds. Each week, one searches five ponds each day for 5 days. The turtles can move among the ponds, so the study population includes the whole suite of ponds. In this case, each week might be considered an occasion. In other words, one would pool sightings over the 5 days. It might happen that turtle Y was seen on both Monday and Friday (it changed ponds). In that case, Y's capture history would report a "1" for occasion 1. Ordinary capture histories never have 2s, 3s, or higher numbers; the animal was either detected (= 1) at some time during the occasion, or it was not detected at any time (= 0).

Detections can be pooled over any length of time. Long occasions increase sample size, but they also sacrifice information content (the animal sighted 12 times during an occasion provides no more information to the model than does the animal sighted once), and long time spans increase the probability that closure will be violated.

Capture probability is casually equated with capture fraction. For a given population lacking behavioral response or individual heterogeneity in capture probability, the detected fraction is the best estimator of average capture probability. Recall that under model M_0 and with an estimated detection fraction (= capture probability) of 0.2, the overall probability of capture history 1001 is $0.2 \times 0.8 \times 0.8 \times 0.2$, or 0.0256. What would it be under M_b? For simplicity, consider a situation in which the probability of first capture is 0.2, as before, but due to trap shyness the probability of subsequent captures falls to 0.1, or 10 percent. In that case, individuals with the history 1001 should occur with the probability of $0.2 \times 0.9 \times 0.9 \times 0.1$, or 0.0162. A capture history 0101 would be $0.8 \times 0.2 \times 0.9 \times 0.1$, or 0.144.

By convention, the probability of first capture in a closed model is represented by *p*; the best estimate of the average value of *p* is designated $\hat{p}$ (pronounced "p-hat"). Recapture probability, *c*, has a best estimator $\hat{c}$. These symbols become seriously confusing when open models are considered, because recapture probability in open models is *p*, not *c*. No first-capture probability can be estimated with an open model. In general, capture probability is designated *p*, but if a distinction is to be made between first and subsequent capture probabilities, the subsequent captures are associated with the parameter *c*.

INPUT DATA TYPE

Although CAPTURE was the leading software for closed model analysis for many years, it has been eclipsed by program MARK. Program CAPTURE organizes models by type (e.g., M_{tb}); program MARK groups models together based on input data type. Input data types are defined by their associated covariates, the presence or absence of latent heterogeneity, and similar attributes.

Recall that individual heterogeneity is said to exist if the probability of capture differs significantly among individuals within the population (e.g., individual 27 is trap shy; individual 42 is not). If the heterogeneity in capture probability can be associated with a class (e.g., juveniles are less trappable than adults), then the data can be analyzed separately by class. Otherwise, the heterogeneity may be idiosyncratic or of unknown origin (i.e., latent). Individual-based heterogeneity can potentially be addressed by an analytical model incorporating an associated individual attribute (quantified by an individual covariate). For example, body size is often used as an individual covariate. If capture probability is associated with body size, individual heterogeneity in capture probability can be modeled explicitly, leading to a better model fit for the parameters of interest. Unfortunately, not all of the analyses available in MARK can accommodate individual covariates; therefore, data streams possessing and lacking individual covariates are separate input types. Furthermore, some models can quantify latent individual heterogeneity using a mixture of binomial curves. The specific input data type choices available in MARK are updated on a regular basis, so investigators should be aware that such choices exist and should address them at the data input stage. Fitting covariates to the analytical model can shed light on the biology of the study organism and improve the precision of parameter estimates. With the "Huggins closed captures" data type (allows individual covariates but cannot model latent heterogeneity as a mixture), for example, an investigator might consider three models: capture and recapture probabilities are the same for all animals ($p=c$); capture and recapture probabilities differ ($p \neq c$) but are the same for all animal sizes; and capture and recapture probabilities (*p* and *c*) are dependent on snout-vent length (SVL), the usual metric of overall reptile size (p_{SVL}, c_{SVL}).

Standard conventions for naming mark-recapture models are available and should be adopted (McCullagh and Nelder 1989; Lebreton et al. 1992). Each model name consists of the parameter names, usually separated by commas, subscripted by letters indicating which main effects or covariates are accounted for in the model. For example, the model designation "p_{SVL}, c_{group}" indicates that the model contains a covariate effect (SVL) on first-capture probability (p) and a main effect (group) on recapture probability (c). To be explicit that a given parameter does not have a subscript, one can follow it with a period, pronounced 'dot.' Thus, a model in which estimates for p and c are separate and constant for all conditions would be called the "p-dot, c-dot" model and written $p.$, $c.$ or "$p_{(.)}$, $c_{(.)}$"). In population biology, upper case N denotes the size of the study population, and lower case n denotes the size of a sample from that population. Lowercase n_i is the sample size on the ith occasion. Thus, if one captured 8 animals out of a population of 43 on day 3, one could state $N=43$, $n_3=8$.

For clarity, it may be useful to cluster strings of parameter subscripts in parentheses. Consider a model in which the p parameter (first capture probability) varies with time and size, but the c parameter (recapture probability) does not; for example, first capture of an animal emerging from hibernation might exhibit a strong size-dependent effect of weather, but once emerged would be relatively insensitive to daily variation in conditions. The model also estimates the population size, N. We might designate the model: $p_{(t+SVL)}$, $c_{(.)}$, N. Such a model would treat the time and size effects additively; that is, the effect of time on a given date would be modeled as a simple addition to the effect on first capture probability of the individual's size. If modeling a statistical interaction between time and SVL (in which the effect of SVL depends on which capture occasion is being considered), the asterisk symbol (*) is used in place of the plus to indicate the interaction: $p_{(t*SVL)}$, $c_{(.)}$, N.

The "t*SVL" subscript shorthand for an interaction glosses over the fact that in such an interaction, one could actually estimate (and indeed the model does so) a simple time effect (t), which is added to the simple size effect (SVL), which is added to the interaction term. Thus, the above model could also be correctly written as $p_{(t+SVL+(t*SVL))}$, c, N. It is useful to keep in mind the expanded version of this expression when one builds interaction models within MARK, as the user must build all three elements (t, SVL, t*SVL) into the model structure. Other software may behave differently. Because MARK can build such complex general linear models, it is an extremely powerful tool, allowing almost unlimited flexibility in constructing insightful analyses of mark-recapture results.

MODEL RANKING

Consider the three models we have chosen to run under the Huggins closed-capture data type: $p=c$, N; p, c, N; and p_{SVL}, c_{SVL}, N. MARK uses a sophisticated algorithm to rank each of these models by the degree to which it fits the data set (see Table 29). The relative merits are quantified in two ways: AIC (Akaike's Information Criterion; see "Assessing Relative Model Fit," below) and AIC weights. Weights are fairly intuitive; the heavier the weight, the better the model. Suppose for example, that the rankings of the above three models are as shown in Table 29. Within the three choices presented, this ranking implies that animal size is a useful feature for understanding detectability (hence a model incorporating this feature has the heaviest weight), and that the relationship between body size and capture probability may differ between first capture and subsequent captures (because the best model has separate parameters for p and c). Even if an investigator is not particularly interested in whether body size affects detectability, this information should be incorporated into the model in order to obtain the best possible overall model fit and therefore the most precise and least biased estimate of other parameters of interest, in this example, population size (N).

In our example, the models ranked first and third have separate estimates for capture and recapture probabilities; the second-ranked model does not. As indicated by the substantial weight associated with the second model, equality between p and c may be a reasonable fit. Consider a model that associates first and subsequent capture probabilities with SVL but forces the two estimates to be equal for a given body size: $p_{SVL}=c_{SVL}$, N. Adding that model to those under consideration might alter the rankings as shown in Table 30. The best model in this case indicates no difference (for a given body size) in probability of first capture and subsequent captures. In other words, there is little evidence for a behavioral response. This might be important for the design of monitoring protocols (for example, open models cannot accommodate behavioral response and therefore would be appropriate only for populations lacking a behavioral response), or it might be just a nuisance parameter.

The addition of model $p_{SVL}=c_{SVL}$, N not only improved overall model fit (it went into first place) but also greatly altered the weights of the other models. The weight of any given model depends on the suite of models with which it is contrasted. No model has any absolute weight. Furthermore, it is always possible that a better model that was not tried exists! One could try every conceivable model and let the rankings

TABLE 29

Models Ranked by AIC Weights, Showing That Detectability Is Related to Animal Size (SVL)[a]

Model	*Weight*
p_{svl}, c_{svl}, N	0.66
$p=c$, N	0.23
p, c, N	0.11

a. AIC = Akaike's Information Criterion; SVL = snout-vent length.

TABLE 30

Models Ranked by AIC Weights, Showing That a Model Associating First and Subsequent Capture Probabilities with Size (SVL), in Which the Estimates Are Equal for a Given Body Size, Best Fits the Data Set[a]

Model	*Weight*
$p_{svl}=c_{svl}$, N	0.76
p_{svl}, c_{svl}, N	0.22
$p=c$, N	0.23
p, c, N	0.00

a. AIC = Akaike's Information Criterion; SVL = snout-vent length.

select the analytical model. The difficulty with this "fishing expedition" approach is the same as for any statistical procedure that is repeated indefinitely: sooner or later one will turn up something that has an excellent model fit, but is so just by chance. If one conducts 100 *t*-tests (with the alpha level set to 0.05) comparing values of two samples drawn randomly from a single population, odds are that five of the *t*-tests will indicate statistical significance (erroneously implying to naïve analysts that the samples were from different populations).

A two-tiered approach to this "fishing" problem seems reasonable. Tier one: *if no one has studied this system before,* it may be appropriate to explore or "fish" for interesting phenomena, recognizing that some phenomena will have a good fit just by chance. Any phenomena identified are prime candidates for the next mark-recapture effort, during which the importance of the identified phenomena will be tested. Tier two: *when testing mark-recapture phenomena,* the suite of models to be tested is identified ahead of time (*a priori*) and only models with good biological justification for consideration are included. If the phenomenon of interest is manifest among the top models in the test context, it is probably a real phenomenon. If it has low rank in the test, it probably appeared in the exploratory sample by chance.

ASSESSING RELATIVE MODEL FIT

Two procedures offered in MARK can be used to compare, or rank, competing models. The first is the likelihood ratio test. If two models differ only in the addition of a single factor or group of factors, the value of adding that factor into the model can be assessed by the ratio of the models' goodnesses of fit. If the computed likelihood ratio is larger than a critical numerical threshold, then the factor's incorporation into the model is justified by the data; if the likelihood ratio is smaller than the critical threshold, its inclusion is not justified. The goodness-of-fit metric in this case is a quantity called *deviance*. Each competing model has an associated deviance (usually computed by the software). Thus, in the likelihood ratio test the deviance of one model (the more complex model) is divided by the deviance of the simpler model. The resultant ratio is compared to the distribution of the well-known Chi-square statistic, with *n* degrees of freedom, where *n* is the difference in the number of parameters between the two models. Consider an example, in which we compare a model that has two parameters ($p=c$, and N) with a more complex model that has three (p, c, and N). If the deviance of the more complex model is greater than that of the simpler model by an amount equivalent to the Chi-square value for significance at $\alpha=0.05$ with 1 *df* (in this case, $\chi^2>3.84$), then incorporating the additional separate parameter, c, for recapture probability does significantly improve the model fit and should be included. The larger the number of parameters added to the model, the greater must be the improvement in fit for the likelihood ratio test to be significant. The likelihood ratio test can be used only to compare models that are nested, that is, where one model is a subset of the other model (if one model includes only factor A and another model includes only factor B, the models are not nested, and they cannot be compared). For this reason, the likelihood ratio test has largely been eclipsed by *Akaike's Information Criterion* (AIC).

AIC is the new standard, and AIC values are now output by virtually all good programs. AIC uses information theory to assess the extent to which a given model extracts the information present in the input data. AIC is the sum of a reward component that reflects the model's goodness of fit and a penalty component that reduces the AIC value in relation to the number of parameters the model used to obtain that fit. Two models with identical goodness of fit but differing number of parameters will yield a better AIC value for the model with fewer parameters; two models with identical number of parameters but differing in goodness of fit will yield a better AIC for the model with the better fit. This is very similar in concept to likelihood ratio tests but is manifest in different values. The concept behind AIC is that you can always get a better fit by adding more parameters, but it is not parsimonious to add parameters just because they cause a better fit. It is better to strive for the best fit that can be obtained with the fewest parameters. Although the computational idea of AIC seems almost simpleminded on first exposure, a wealth of statistical theory and experience supports its mathematical structure.

The statement above, that AIC is the difference between a reward component and a penalty component is correct, but it was stated in reverse order for ease of comprehension. AIC is actually a negative measure of the reward component added to a positive value for the penalty component (or the penalty minus the reward). As a result, the best AIC is the lowest value from among several choices. For example, suppose that the three-parameter model mentioned above (p, c, N) has an AIC of 1218.4 and the two-parameter model ($p=c$, N) has an AIC of 1222.2. In this case, the preferred model would be the three-parameter model (lower AIC value).

The degree of inferiority of a model is generally expressed as ΔAIC (pronounced "delta AIC"). In comparing just the two models above, the ΔAIC of the three-parameter model would be, by definition, zero, whereas that for the two-parameter model would be the numerical difference between the lowest model and the focal model, or 3.8. As a general rule, models with ΔAIC values of 2 or less are considered to be virtually indistinguishable in merit, whereas a model with a ΔAIC of 10 or more would be considered too poor for serious consideration compared to models with higher rankings. Models with intermediate values of ΔAIC (2-10) are arguable. Thus, in the above case, the two-parameter model would be worthy of consideration, but it would be considered definitely inferior to the three-parameter model. Models in MARK are ranked by AIC and are weighted in accordance with ΔAIC (larger ΔAIC models have lower weights).

The current version of MARK uses a variant on AIC that is known as QAIC_c, which includes two corrections, as indicated by the leading Q and the following c. The Q stands for quasilikelihood, a correction for overdispersion in the data. Data can be overdispersed for several reasons, the most likely of which occurs among social species, which flock or otherwise tend to behave similarly. Specifically, when one individual animal gets caught, the others of the same group are more likely to do so; when one leaves the area, the others are likely to do so, and so forth. This leads to multiple animals exhibiting the same or similar capture histories. It also implies that counting each of the individual capture histories as independent samples overstates the sample size and therefore underestimates the true variances. The term overdispersion is a bit counterintuitive, as we usually think of spatial clumping as a result of under dispersion; in this case, however, the term overdispersion indicates a distributional anomaly that is addressable using QAIC. The amount of overdispersion is easily estimated by a computer (it is routinely reported by the software); it reduces the reward component of AIC proportional to the amount of overdispersion, yielding QAIC, which is believed to be more representative of the true value of the model. If there is no overdispersion, then QAIC = AIC. In my experience, correction for overdispersion makes little difference for most reptiles, which tend to behave independently. Social species, such as territorial lizards, may show greater statistical overdispersion in their capture histories, but MARK can make the correction automatically.

MARK makes a second small adjustment to AIC for effective sample size (see Cooch and White 2005 or the help files in MARK); it is denoted by the subscript "c" at the end of $QAIC_c$. Unfortunately, the overdispersion parameter in MARK is designated c as well, leading to potential confusion with the effective sample size correction ($QAIC_c$), as well as recapture probabilities (*c*). When reading mark-recapture output, pay attention to which c is being reported. Regardless, it seems reasonable always to correct for small sample size. Therefore, $QAIC_c$ is the currently preferred measure, as it automatically incorporates the various corrections.

$\Delta QAIC_c$ has an absolute meaning (models with values closest to zero are most comparable), but $QAIC_c$ has no meaning in the abstract, as its value scales with the sample size. Accordingly, one cannot compare $QAIC_c$s among models that have different sample sizes; indeed one cannot legitimately compare $QAIC_c$s between any models that differ in input data set. MARK is set up to compare $QAIC_c$s (i.e., compute $\Delta QAIC_c$) only among models that stem from the same input (which is why MARK, as opposed to CAPTURE, groups models by input data type). This should be kept in mind in choosing which data type to use for creating a model set in MARK. All of the models that you wish to compare should be analyzed in one data type, if you wish to have MARK compute $\Delta QAIC_c$, weights, or average parameter estimates among models (see next section).

ASSESSING ABSOLUTE MODEL FIT

There are various ways in which a given model can have the best AIC or $QAIC_c$, but still not fit the data very well. Sometimes, causes of poor model fit can be revealed by goodness-of-fit tests performed by MARK using the embedded program RELEASE (see Appendix II). My experience, however, is that the tests are fairly weak when used with typical reptile data sets, which tend to be small. They are robust with large, well-behaved data sets, such as those obtained from large-scale fisheries marking projects. The RELEASE goodness-of-fit tests should always be performed and reported in papers citing mark-recapture results, but little importance should be attached to failures to reject the null hypotheses with small data sets.

MODEL AVERAGING

The weights associated with each model have a special value when the most appropriate model overall is uncertain. If the weight resides almost exclusively in one model (say, the top model has a weight of 0.93), it is reasonable to conclude that the model is the best one and should be used to obtain parameter estimates (e.g., population size). If several models have roughly equivalent weights, however, it is difficult to know which of the parameter estimates they have generated would be best. MARK has a lovely feature that averages the population estimates from several models, with the estimates weighted according to the models' relative merits (weights). This process is known as *model averaging,* although it is the parameter estimates that are averaged (among models), not the models themselves. When model averaging, MARK adjusts confidence limits to reflect not only the numerical uncertainty of the parameter estimate but also the uncertainty about which model is the best choice. Consider two models of nearly equal fit (weights 0.54 and 0.42). One might estimate a population size of 110 (85–148) and the other of 92 (71–122). In this case, instead of trying to choose between the two population estimates, it might be better to model average, using the MARK-provided weighted average estimator and confidence limits. These values will *not* be the same as those computed by simple averaging.

Confidence limits produced by MARK are not usually symmetrical. That is, the range above the best estimate is often larger than the range below. For example, in the example 92 (71–122), the lower value is 21 below the best estimator (= 92–71), whereas the upper value is 30 above it (= 122–92). This difference derives from the fact that the software is estimating *p* and *c,* which can only have values between 0 and 1. If the best estimator of *p* is 0.05, for example, it would be unreasonable to have symmetrical confidence limits of –0.03 to 0.13. A negative capture probability is not meaningful. Therefore, the software constrains all appropriate values to the range 0 to 1 by making each increment count more when approaching the outer bounds. Instead of a linear function between 0 and 1, the program uses a variety of s-shaped relationships. Thus, the confidence limits for a $\hat{p}$ of 0.05 is asymmetrical (e.g., 0.008–0.119).

Related to this asymmetry is MARK's calculation of *maximum likelihood estimators* (MLEs) rather than means. These are the values that are most likely to be true, but not necessarily the ones that are in the middle of the probability distribution (as is a mean for a symmetrical distribution). Explanations of the iterative methods needed to compute an MLE can be found in statistics books, although in almost every case related to mark-recapture, MARK automatically computes the MLE. Statisticians highly favor MLEs because they are optimal for large samples in three senses: (1) they are asymptotically normally distributed, (2) they have asymptotically minimal variance, and (3) they are asymptotically unbiased.

DESIGN MATRICES

Consider the simplified situation in which all of the factors influencing MARK parameters are linear; the desired output would be estimates of the slopes and intercepts of each linear relationship. For example, suppose that detectability is constant over time but linearly related to the size (snout-vent length, or SVL) of the reptile. The MARK user wishes to know the position (slope and intercept) of the linear function relating detectability to SVL. The slopes and intercept values are collectively termed *coefficients.* If the functional relationship is nonlinear (i.e., there are squared, cubed, or other more-complex terms), there will be additional coefficients of interest, with the higher order coefficients used to characterize the shape of the curvilinear function.

The numerical structure of MARK requires that coefficients for each parameter of interest first be fit on a standardized scale (zero = no effect), at which point they are called *beta coefficients* (i.e., estimates of the *beta parameters*). Beta coefficients (often colloquially called beta parameters) are not in the same units as the original data (e.g., SVL may be measured in millimeters, over the range 600–1,000); when converted to the original units, the coefficients are termed *real coefficients* (i.e., best estimates of "real" parameters). Consider a simple example in which detectability is associated with the size of the reptile. The size (SVL) of each individual will have been entered into MARK as a covariate in units of, say, mm. Suppose that the mean capture probability (= detectability) increases 0.02 for each 100-mm increase in SVL. MARK will estimate a beta parameter for SVL that reflects the strength and direction of the influence of SVL on detectability. Because the association is positive (increasing SVL is associated with increasing rather than decreasing detectability), the beta value will be positive, say, 3.42. The magnitude of beta reflects the strength of the relationship between detectability and size, and the confidence limits of beta express the range of strengths that the relationship might have. If the 95 percent confidence limits of the beta value broadly overlap zero (one end of the confidence limits is negative and the other end positive; e.g., –0.3 to 0.7), one would have little confidence that the phenomenon (in this case, size influencing detectability) was real. In any case, one would be unjustified in claiming such a relationship on the basis of the data at hand. A larger sample size might reveal that the phenomenon is, in fact, real, but with such a confidence range (beta: –0.3 to 0.7) one would have little confidence that the phenomenon was real based on the sample size that one had.

To determine the slope of the relationship between detectability and size (in our example 0.02 for each 100 mm), one needs to know the real parameter coefficient, which is related to the beta coefficient by a mathematical function called a *link function* (see "Link Function," below). MARK provides for a variety of link functions to be used (user specified), but in most cases one can rely on MARK to chose the correct link function and to properly back transform the beta coefficients into real coefficients on the basis of the link function that MARK used. Real and beta parameters are both useful to the researcher (although for different reasons), and both sets of coefficients are reported by MARK. However, it is vital that readers understand that the *design* of MARK models is handled through arrangement of beta parameters (don't fear; I will explain).

Suppose that one is building a model for six trapping occasions (= days) that recognizes that it rained on occasions 2 and 3, which appeared to severely depress initial capture rates on those days. *Re*capture rates were not affected on the rainy days. The full model in this case (p_{day1}, p_{day2}, p_{day3}, . . . p_{day6}, *c*, *N*) contains eight parameters, six (one first-capture parameter for each day) for the time effect on *p* and one parameter each for *c* (recapture probability was the same every day) and *N* (abundance was assumed to be constant throughout the 6 days). However, *p* may not have been unique on every day; it may have differed only between rainy and sunny days. If the six *p* parameters can be reduced to two without appreciably lowering the overall model goodness of fit, AIC will improve by virtue of the reduced number of parameters. To set up such a model within MARK, one assigns a beta value to account for the capture probability difference associated with rainfall (i.e., the first capture probabilities on days 1, 4, 5, and 6 are the same and have a different beta from the capture probabilities for days 2 and 3, which are also the same). Two other betas are needed, one each for time-constant parameters *c* and *N,* for a grand total of four ($p_{\text{days1,4,5,6}}$, $p_{\text{days 2,3}}$, *c, N*) rather than the eight of the full model.

To convert a MARK model from the full version (eight parameters) to the reduced version (four parameters), one *constrains* some of the original eight to be identical to each other. This constraining is accomplished by assigning a single beta parameter to represent more than one of the original parameters. In our example, one assigns a single beta to represent the capture probability on 4 days (1, 4, 5, and 6); a different beta represents the capture probability on both days 2 and 3. The layout of these constraints follows a convention established for MARK (and many other programs) that is called a *design matrix*. A design matrix is a standardized (matrix) representation of beta parameters. A simple example for the above case can help illustrate the arrangement (see Table 31).

This matrix may not be exactly what one envisioned from the previous discussion, but as we shall see, it functions exactly as intended. One of the rules of design matrix construction is that there must be one beta coding for each parameter overall (this "anchoring" beta for each parameter is called the *intercept*). In the above example, betas 1, 3, and 4 provide intercepts for *p, c,* and *N,* respectively. When back transformed to a real coefficient, β_2 represents the capture probability difference between the sunny days and the rainy ones. β_2 could just as easily have coded for the capture probability difference between rainy days and sunny ones in the opposite sense (i.e., a 1 would have appeared in rows 1, 4, 5, and 6, with zeros in rows 2 and 3). It makes no mathematical difference which formulation is used, but the user must keep track of which is used to interpret the resulting beta parameter values. In the formulation illustrated above, the estimated real values for capture probability on the 4 sunny days was derived solely from β_1, whereas the real value for the 2 rainy days was a linear combination of betas 1 and 2. Because first-capture probabilities for all 4 sunny days are derived from the same beta (β_1), they are constrained to be the same. Similarly, first-capture probabilities for the 2 rainy days are constrained to be the same because both are taken from the mathematical difference between β_2 and β_1. It is not necessary (or even desirable in this case) to have a separate beta representing sunny days only (1, 4, 5, and 6), as those days are uniquely represented by the intercept (β_1) *without* β_2.

The beta structure not only facilitates the construction of the general linear model for each parameter, but it also facili-

TABLE 31

A Sample Design Matrix, or Standardized (Matrix) Representation of Beta Parameters

	β_1	β_2	β_3	β_4
$p_{(\text{day1})}$	1	0	0	0
$p_{(\text{day2})}$	1	1	0	0
$p_{(\text{day3})}$	1	1	0	0
$p_{(\text{day4})}$	1	0	0	0
$p_{(\text{day5})}$	1	0	0	0
$p_{(\text{day6})}$	1	0	0	0
c	0	0	1	0
N	0	0	0	1

tates the iterative calculation of each maximum likelihood estimator (MLE). The complexity for the user is that some program output is in terms of betas, rather than real values, and the back transformation from betas to real values requires inverting the link function.

LINK FUNCTIONS

A *link* is used to connect the beta parameters to the real parameter values. A link function expresses the shape of the mathematical relationship between betas and reals. There are presently six possible link functions for use with MARK. One common link is the logit link, which is related to the real value as $\frac{\exp(X\beta)}{1+\exp(X\beta)}$, where X is the user-provided design matrix (for example, the eight row/four column matrix presented in the last section), and β is the vector of beta coefficients estimated by MARK. (Readers unfamiliar with this matrix notation should consult an introductory text on matrix algebra, or see the appendices in Williams et al. 2002 and MacKenzie et al. 2006). The "exp" function in this equation is the famous constant "e" raised to the power indicated in the subsequent parentheses, in this case, the product of the matrix and the vector (i.e., $X\beta$). The back transformation can be done easily with a spreadsheet such as Excel, but in most of the straightforward cases MARK will do it. Not every link will work for every situation. MARK generally chooses the correct link, although an investigator can choose the link for situations that require a specific link (see Cooch and White 2005). Examples of manual back transformations can be found in help files on the MARK website.

Because modern statistical approaches to the analysis of mark-recapture data are complex and require iterative methods, they are routinely performed only on a computer. For example, models incorporating individual heterogeneity use the statisticians' "jackknife" procedure iteratively to divide the data set into progressively finer subsets to find the optimal number of different capture probabilities to fit the data. Although one can carry out the jackknife procedure on a calculator, the process might drive him or her crazy. In the preceding paragraphs I have identified several other mathematical operations that are best handled by a sophisticated computer program such as MARK: asymmetrical confidence limits, maximum likelihood estimators, weighting, link functions, goodness-of-fit tests, estimation of overdispersion, and computation of QAIC_cs. For these reasons, few people now attempt mark-recapture analysis by hand.

Simple-to-use software is a magnificent tool in the hands of those that understand it. Lack of space prevents me from presenting all the information necessary for running program MARK, however. The most important first steps are to recognize and understand the concepts behind the program and to interpret the values reported in mark-recapture literature properly. The information in this chapter and the MARK manual (Cooch and White 2005) should enable readers to do that. The structure of the program will no doubt change as additional useful features are added. I strongly encourage prospective users to obtain research papers and manuals associated with the program (available from the MARK website) and to develop a complete understanding of the power and limitations of each feature of the program. Users should then be able to design their own studies and analyze their findings correctly.

Absolute Estimators IV: Open-Model Mark-Recapture

Open-model analysis of mark-recapture allows for closure violations by estimating the number of animals that have left the population after having been marked. Under some circumstances, such as when additions to the population are by birth and the physical appearance of young animals is distinct, one can also estimate the recruitment rate. These additional parameters may be nuisance values if, for example, the goal of the study is to estimate population size. On the other hand, they may be the goal of a study if one wishes to estimate the rate of disappearance of marked animals under a specified harvesting scheme.

For estimating population size, closed models are more precise, flexible, and intuitive than open models. Closed models can easily accommodate behavioral responses to trapping as well as individual capture heterogeneity, which open models have not been able to do. However, a relatively recent innovation allows Pledger's mixture paradigm for capture heterogeneity to be applied to open models (Pledger et al. 2003; see also Pledger and Efford 1998). Pledger's procedure decomposes each complex detectability distribution into a mixture of simple binomial distributions. Given the criticism of the mixture paradigm for closed models (Dorazio and Royle 2003, 2005; Link 2003, 2004; Pledger 2005), it remains to be seen whether the open-model application of mixtures performs well with empirical data. One would always use a closed model rather than an open model for population size estimation if closure was assured. As indicated in the preceding section on closed models, closure can sometimes be approximated by shortening the time interval to a point where negligible emigration occurs. The robust model (see "Absolute Estimators V," below) uses short intervals, which may provide closure, in combination with open-model analysis of longer time intervals.

ASSUMPTIONS

All mark-recapture models, including open models, assume that marks are not lost, are read correctly, and are recorded properly. Standard open-model formulations, often called the Cormack-Jolly-Seber (CJS) model or simply the Jolly-Seber method (in honor of early developers), estimate capture probability *conditional on first capture.* In other words, these analytical models actually estimate recapture probability of animals known to be present in the population because they were marked there. In essence, basic open models track the fate of animals already marked. In an open model, an animal can remain in a population with a probability p of being recaptured, or it can leave the population (by death or emigration), as measured by phi (Φ), the statistical "survival" parameter. Survival is in quotes because a true survival rate is (1 – death rate). Open-model survival is (1 – [death + emigration] rate). In the sense it is used in an open model, phi is often called *apparent survival,* although it is better thought of as "probability of remaining in the population." Open models measure statistical survival (the fraction remaining in the sample pool) rather than literal survival. To distinguish true survival from statistical survival requires a way to distinguish emigration from death.

To estimate population size from an open model, one needs to assume that first and subsequent capture probabilities are equal—that is, that animals that have never been caught have the same probability of being captured as animals that have been caught previously. This implies the absence of a behavioral response (trap shyness or trap happiness), a key assumption that is likely reasonable for many reptiles.

Unlike closed models, open models generally cannot accommodate latent individual heterogeneity in capture success. Because population size is derived from the estimate of capture probability, uncategorized capture heterogeneity biases estimates of population size. Many reptile species will exhibit individual heterogeneity in capture success, but they can nonetheless be accurately analyzed with an open model if the source of the heterogeneity can be recognized or accounted for through individual covariates. If one knows, for example, that small individuals are harder to detect than large ones, one could stratify the analysis by animal size (treat the different size classes as separate analytical groups) or include snout-vent length as a covariate. Stratification of a sample requires that each stratum have an adequate sample size, a hurdle that can be a significant one for some reptile species. Confidence limits for strata with small sample sizes tend to be broad.

The assumption of no behavioral response can also be relaxed in some situations. Suppose that marking is traumatic for the animals and causes them to hide for a few days following first capture only. Recapture might be less traumatic, since additional marking is not needed. In that situation, probabilities for initial recaptures and for recaptures more than, say, 3 days after marking could be distinguished (given separate parameters in the design matrix). A distinct (reduced) recapture probability could be assigned to animals in the first and second days after original marking. This special type of behavioral response can be handled by an open model. As a subset of recaptures could be used to estimate first capture probability, such a model allows abundance estimation.

Although emigration rate cannot ordinarily be segregated from death in open-model analyses, temporary emigration is a special situation. In essence, an open model treats a temporary emigrant as if it were present the whole time but was very hard to capture. This assumption violation biases the estimate of *p,* capture probability, but may not greatly bias an estimate of apparent survival. Depending on the covariation of *p* and phi, and the purpose of the analysis, a bias in capture probability may not be important.

SOFTWARE

MARK (Cooch and White 2005) is the premier software for the analysis of open populations. Many types of input data exist for MARK under various open-model situations. Most of these types rarely apply to reptiles (e.g., band recoveries, tags turned in by hunters, etc.), but in certain special situations they could be invaluable. MARK has a full array of age-based models. These are most often used for birds that are banded only as fledglings, with the result that the age of each individual is known from the interval since marking. Ages of only a few reptiles can be inferred so easily, but some highly seasonal reptiles have distinct cohorts of young-of-the-year (YOY), probable ages will be known for very long-term marking programs with some reptiles, and some species can be aged by skeletochronology or other objective aging techniques.

The general structure of MARK is the same for both open models and closed models. Analytical models within a data type are ranked by AIC, weighted, and so forth. One may model-average to obtain parameter estimates incorporating the uncertainty regarding model selection. Nearly unlimited opportunities exist for adjusting parameter values for different subgroups; individual covariates are readily applied, as are various constraints on parameter values.

MARK is structured to estimate population size under a "classical CJS" model in which population size is estimated independently for each capture occasion. This may be the correct formulation in many situations, but the resultant population estimates are, therefore, based on very little information and are correspondingly imprecise. If capture probabilities are very large (say, >0.5), one can obtain reasonable estimates of population size for each capture occasion, but few reptile species have so large a mean capture probability. The preferred solution to this dilemma is to use the robust model, described in the following section. The robust model requires multiple capture occasions over an interval of time so short that closure can be assumed for the closely spaced captures.

An alternate approach with special value for many reptile studies is to use an open model but base the estimate of $\hat{p}$ on multiple occasions. Suppose the study reptile can be sampled consistently (little temporal variation in capture probability). With samples from enough occasions it is possible to obtain a relatively precise estimate of capture probability $\hat{p}$, even for capture probabilities (e.g., 0.01) that are much too low for population-size estimation with conventional open-model analysis. If one is comfortable with the assumptions of neither latent individual heterogeneity nor any behavioral response, one can estimate population size $\bar{N}$ by the relationship $\frac{\bar{n}}{\hat{p}}$ where $\bar{n}$ is the mean number of captures per occasion (both marked and unmarked) and $\hat{p}$ is the capture probability estimated as indicated above. The confidence limits associated with capture probability provide the confidence limits for population size ($\bar{n}$ is not estimated but known from the input data set, so it has no sampling error).

Absolute Estimators V: Combining Open and Closed in the Robust Model

The robust model takes advantage of the best features of both open and closed models by including both in a single analysis. Not only can one obtain more information (e.g., temporary emigration) from a dual model than either model alone provides, but one can also analyze long-term trends in population size (lambda) that would otherwise require a meta-analysis of multiple mark-recapture estimates. Lambda (λ) is a rate that is the ratio of a later population size over an earlier one. The rate aspect of lambda implies that empirical values of lambda need to be referenced to a definite time interval, often a year or a generation. However, for any time interval, populations with a lambda > 1 are increasing, those with a lambda = 1 are stable, and those with a lambda < 1 are declining. Because population sustainability can be interpreted independent of time interval, lambda is often discussed in the abstract; however, empirical values of lambda need a specified time interval for a rate of change to be interpretable. Lambda is often the key parameter that is modeled in life table analyses or population viability analyses (see those sections below).

Data for a robust model are collected in bouts. A bout is a time interval short enough for demographic and geographic closure during which multiple samples are collected. Bouts are separated by long time intervals called primary periods; the short time intervals (treated as infinitely short) within bouts that separate sampling events are called secondary periods. The secondary periods are analyzed with a closed model, and the primary periods are modeled as open following the model protocols described above. Three additional parameters in robust models are temporary emigration probability, γ'; temporary emigration probability of individuals that were not present during the immediately preceding secondary period, γ''; and lambda, the ratio of later over earlier population size. The temporary emigration estimates are possible because the capture probabilities estimated from the secondary periods allow one to judge the probability that an animal was present but undetected during a secondary period. Because temporary emigration can only be known to be temporary in light of subsequent detection, some of the parameters cannot be estimated for the first, second, next to last, and last primary periods.

Under favorable circumstances, temporary emigration probabilities could be used by reptile ecologists to quantify participation in seasonal breeding activities that may be skipped by some individuals. For example, if the probability of adult female presence in a study area at oviposition season varies about two-fold among seasons, one may surmise that about 50 percent of adult females leave the study area to oviposit. Alternately, if one's study area included just the oviposition site, one would expect adult males and juveniles to be absent throughout, with temporary appearance of the females at the time of oviposition. Normally, the breeders would have to behave in some recognizable way (become aphagic and therefore untrappable using food-baited traps, leave the study area, etc.) for this participation in optional breeding to be quantified by this method. For example, it may be possible to estimate the proportion of biennial breeders in a population accurately by obtaining accurate measures of capture probability for each year under the robust model.

Multi-stratum models are mark-recapture models that sample animals in two or more different places or states (e.g., breeding vs. nonbreeding). Imagine a situation in which animals are monitored in two or more places among which they occasionally move. An animal that disappears from one place may not have died; one can estimate the probability that it was present at the other site based on mark-recapture evidence obtained there. Multi-stratum models are especially appropriate for migratory species, but they can also be used to analyze movements among study plots that are smaller than the activity ranges of individuals. For example, Savarie and colleagues (2001) used a multi-stratum model to quantify the amount of movement between adjacent treatment and experimental plots in a study of snake control techniques. The strata sampled with a multi-strata model need not be adjacent; the model will estimate the probabilities of movement between strata regardless of their geographic location. The between-strata movement probabilities estimated by multi-strata models sum to 1, indicating that movements from a measured stratum to an area beyond the sampled strata are not estimated. Thus, the amount of movement among strata revealed by a multi-stratum model sheds light on the likelihood that closure between the sampled strata and unsampled localities is plausible, but a multi-stratum model does not measure the degree of closure violation directly. MARK has several multi-stratum model data types, which should suffice for most reptile applications.

Estimating Density from Abundance and Area

All of the absolute estimates that I have discussed estimate abundance rather than density. But without a measure of the area involved in a given abundance estimate, there is no basis for comparing two abundances. In contrast, abundances of an isolated population that is likely to remain so can be compared through time. The vague geographic boundaries of the population in most studies will change over time (although often the change will go unnoticed). Thus, for long-term monitoring programs, interspecific, or intersite comparisons, density is a far more useful statistic than abundance. To convert abundance to density, one needs to know the area occupied by the sampled population. This problem has not received much attention from statisticians and does not seem to have a definitive solution. Investigators usually decompose the study area into the activity ranges of the contained individuals. Some individuals will reside entirely within the trapping area and are, therefore, unquestionably part of the sample population. Individuals that reside on the periphery of the trapping grid whose activity ranges extend beyond its perimeter are a problem.

One could compute the area of the population as the trapping area plus the peripheral activity ranges (assuming that only individuals of the study population occupy those peripheral areas). This solution may not work well for reptiles, however, as most computational formulae are based on movement patterns characteristic of mammals and birds, rather than reptiles. Because the software is written with endothermic behavior patterns in mind, it is fruitful to consider the ways in which reptile behavior differs from that of endotherms.

Endotherms are characteristically hyperactive, visiting most of their activity area during the course of a single trapping occasion. The placement of traps for mammals reflects this. A practical rule when trapping small mammals is to place from two (Wilson et al. 1996) to four (White et al. 1982) trapping stations in each activity range. As the target population should be in the range of 100–200 animals, and the sexes often have overlapping home ranges in rodents, a trapping grid of about 100–200 traps is typical. Consider a simple numerical example: suppose that one male and three females occupy a typical home range (1 hectare in size) and that four traps are placed in each hectare. For such a study area to target a population of 100 animals (25 nonoverlapping home ranges of 1 hectare), the trap grid would need to cover 25 ha. Thus, trap grids for endotherms tend to be large in relation to a target species' activity range, and relatively few endotherm activity ranges straddle the trap-area boundary. A rule of thumb is that trap grids should cover an area *at least* four to six times the size of the average home range of the target organism (Wilson and Anderson 1985); in the simple example given above, the trap grid covered 25 average home ranges.

In contrast, reptiles often visit only a small part of their activity ranges during each 24 hours. Thus, in order to ensure reasonable capture success, many traps must be placed within each animal's activity range. Furthermore, because activity is infrequent and/or irregular, reptile capture probabilities are often intrinsically low, leading researchers to set the duration of an occasion to days or weeks rather than hours. Finally, the average locomotion rate of many reptiles is low, so that large portions of an individual's activity range may not be visited for long periods. In some cases, the full activity range of each individual may be many times larger than a practical trap grid.

Thus, many or all of the individuals in a trapped population may have activity ranges that straddle the trap area boundary.

It is vital to distinguish between the mean sizes of the activity range over an occasion and the activity range over the entire trapping period. For example, a typical snake may range over 0.3 ha in the course of a night (= occasion), and over 10 ha during a 3-week trapping period. Because population size is estimated from $\hat{p}$, or the capture probability per occasion, the key activity range for our present purposes is the one associated with an occasion. If the activity range per occasion is the same size as the activity range per trapping period, then the population is geographically closed; that is, the animals trappable on the first night will be the same individuals that can be trapped on the last night (and all nights in between). If the activity range per occasion is substantially smaller than the activity range per trapping period, some individuals on the periphery of the trapping grid will drift out of the sample area over the course of the trapping period, others will drift in, and the population will not be closed geographically. Differences between these two measures of activity range can help to guide selection of the proper analytical model for the data.

For a sampled population to be closed, the distance between adjacent traps must be less than the diameter of the smallest activity range per occasion. If trap spacing is wider than this, some individuals in the interior of the trap grid will not enter the trappable area during a trapping occasion, and these individuals will effectively immigrate into the detectable population when they drift into the vicinity of a trap site. In essence, the sampled area will be holey like a slice of Swiss cheese; not only will closure be violated, but it may also be impractical to determine the size of the sampled area (solid cheese). Although setting the intertrap spacing exactly at the diameter of the smallest activity range per occasion would suffice to prevent interior holes, it is best to provide a margin of error by placing the traps somewhat closer together. The classic recommendation is to have at least two traps per activity range (or set the intertrap distance at the radius of a minimum activity area per occasion). This criterion has not been tested for any reptile, but seems reasonable. If traps are inexpensive to deploy, even smaller trap spacing may be better.

The choice of spacing will also depend on the trap/animal interaction. Single-capture traps, such as trip traps, should be set at a closer spacing than multiple-capture traps, such as pitfalls, that continue to function once occupied. Most reptile traps can capture many individuals without being reset, but certain types of traps may be less potent if they are occupied by a prior captive. For example, a struggling stuck individual may forewarn some species of lizard of the presence of a glue trap. Some species may refrain from occupying a coverboard that already houses a larger conspecific or predator. Another important consideration is the overlap among areas. Traps set for species with nonoverlapping activity areas can be relatively widely spaced, as each trap has the potential of capturing only a single individual (or perhaps one of each sex); additional traps may be needed for species whose activity areas overlap extensively, to ensure that trap success is not limited by the inadvertent warning action of previously caught individuals.

The general rule of thumb for quantifying the *effective area of a trapping grid* is to add to the bounded area the area of a strip that is as wide as the radius of the average activity area per occasion. Consider, for example, a 4×4 grid of traps spaced 25 m apart (Fig. 94). The *bounded trapping area* is 75×75 m, or 0.56 ha. The *nominal trapping area* is the area that assumes that each trap "covers" an area equal to the intertrap spacing, or 25×25 m. In this case, the nominal area is 100×100 m, or 1.00 ha. If individuals of the target species are drawn to a trap because it has a large attraction radius, it may be more appropriate to consider the effective trap area to be the nominal area. If the trap has a small or negligible attraction radius, the bounded area may be more appropriate. Let us suppose that

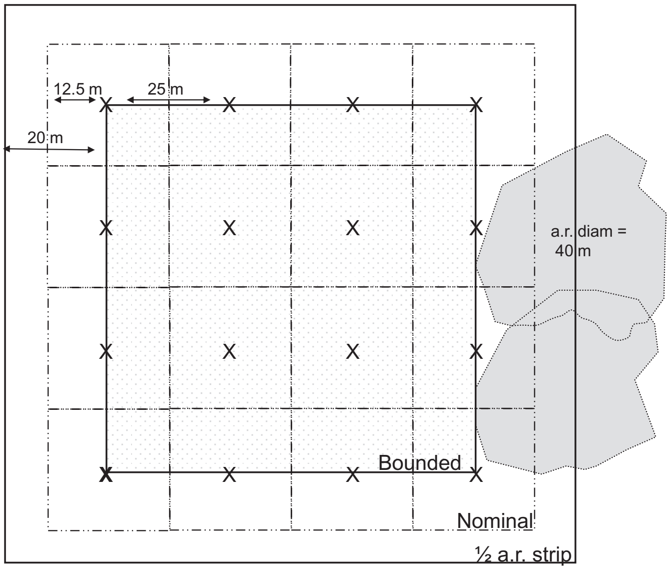

FIGURE 94 Calculation of the area of the trapping grid. "X" denotes trap placement. Drawn boxes are the bounded area (inner solid line), the nominal area (dot-dash line), and the effective area (outer solid line), which was determined by adding a strip of a width equal to one-half the activity range diameter around the bounded area. Representative activity ranges are shown by the shaded polygons.

we are trapping daily for a species whose average daily activity area has a diameter of 40 m (radius 20 m). Adding a 20-m strip around the outside of the 100-×100-m nominal trapping area would produce a sample area of about 140×140 m, or 1.96 ha. A 20-m strip added to the bounded area would create an area of 115×115 m, or 1.32 ha. Thus, depending on which area we use, the effective trapping area may vary nearly fourfold in size (0.56 to 1.96 ha)! Tests have not been conducted to determine if the boundary strip should be added to the nominal area or the bounded area; usually it is added to the bounded area. If MARK had indicated a population size of 25 individuals for such a trapping grid, density estimates based on the four measures of trapping area (12.8/ha, 17.4/ha, 25/ha, and 44.6/ha) would be quite different. Discrepancies of this magnitude are common in reptile studies, and they underscore the potential for bias associated with sampling area. Unfortunately, no one has yet validated any technique for definitively estimating the sampled area for any reptile mark-recapture program. This problem affects all sampling methods, including nontrap methods such as visual encounter surveys.

Occasionally, activity ranges of mammals are estimated based on the mean maximum distance moved (MMDM) between capture sites over the entire trapping period (Wilson and Anderson 1985). This approach may have merit for species whose activity range per occasion is similar in size to their mean activity range over the entire trapping period, but it is likely to be influenced by sample size. That is, as the trapping period is lengthened, additional information on location is obtained, leading generally to progressively larger mean maximum distances moved. Any metric, such as MMDM, that is a function of sample size is a suboptimal choice for estimating a fixed value (such as mean sample area per occasion). Nonetheless, in a simulated movement of a mammal, MMDM performed tolerably well, estimating an actual sampling area that was about 80 percent larger than the bounded trapping area (Wilson and Anderson 1985).

A recent innovation in estimating trap grid area is DENSITY (see Appendix II), a procedure and software invented by Murray Efford (Efford 2004; Efford et al. 2004, 2005). He took a method used to accommodate individual heterogeneity in detectability for open population models (Pledger and Efford 1998) and modified it to model animal movement patterns to obtain geographic patterns of captures that match those obtained empirically. The method is somewhat akin to MMDM in that it adjusts the effective trap area on the basis of the obtained geographic pattern of trap captures. The effective trap area is inferred to be the correct one for the animal movement pattern that was modeled. In my experience, program DENSITY produces plausible absolute population density estimates, but it requires relatively data-rich input streams. Sparse data sets, such as those typically obtained with reptiles, may not produce estimates. However, very little literature on the validity of DENSITY in the field is available, so no consensus has emerged as to the soundness of this approach. I encourage readers to report the results of reptile studies using DENSITY, especially field validation trials.

One often overlooked complication associated with deriving a density estimate from estimates of abundance and area is that area is estimated with some uncertainty. If one knew the area and measured it without error, one could simply divide the abundance confidence limits by the associated area. Consider a simple numerical example in which the lower and upper confidence limits (LCL and UCL) on the MLE (*maximum likelihood estimator*) of 25 individuals in the population are 20 and 35, respectively. If the area were known without error to be 0.56 ha, the associated LCL for density would be 20/0.56 ha = 35.7/ha, and the density UCL would be 62.5/ha. But we cannot assume measurement without error, and we have a great deal of uncertainty about which area measurement most accurately reflects the true sampling area. Thus, the real 95-percent confidence limits on our density estimate are wider than 35.7 to 62.5 by an unknown amount.

In the absence of validated procedures for sample area estimation, it is doubly desirable to minimize the size of the potential area errors. Probable errors in sample area estimates can be minimized by making the trapping area as large as is practical. Larger areas are less influenced by uncertainty over the width of the boundary strip. Reptiles that are large enough to carry transmitters can be radiotracked concurrent with mark-recapture to provide a detailed record of the size of the activity range per occasion. If there is substantial classifiable heterogeneity in activity range size, it may be best to stratify the sample by whatever feature is associated with the heterogeneity (e.g., age or size/juveniles versus adults). That will reduce errors of sample area estimation and limit them to the affected strata. For example, if juveniles have much smaller activity ranges than mature individuals, preparation of a separate density computation for juveniles and adults will minimize errors in sample area estimates.

Obtaining the Best Point Estimates of Density

The accuracy (reduced bias and increased precision) of a population estimate can be increased in various ways. Some of the more important actions that an investigator can take with respect to data collection and data analysis follow. A researcher should always

1. Strive for the highest possible detection fraction. Biases that creep into population estimation, including closure problems, individual heterogeneity, behavioral response, and so forth, have a greater effect on data sets with a low detection fraction. Consistency of detection effort is also important (especially for removal sampling), but in most cases, less so. Increasing the number of searches, using a stronger attractant for traps, increasing the density of search lines, and similar measures can help to increase the detection fraction.
2. Aim for a representative sample. Capturing only large animals, for example, may be easier and more efficient, but selecting the sample or the site for logistical reasons limits the generality of the results.
3. Minimize tag/mark loss. Obviously, no loss is best, but that is rarely achievable in long-term reptile studies. Double- or triple-tagging or marking may be needed initially to verify that retention rate is near 100 percent. Loss of a solitary tag not only causes the statistical death of the previously tagged individual but also results in the appearance of a statistical immigrant in the population. Data management failures (e.g., erroneous transcription of a tag number) can produce the same double error and should be painstakingly avoided.
4. Search for evidence of closure failure. A classic error that researchers make when most animals are recaptured close to the original point of capture is to

assume that the mobility of the species is low. In fact, such a capture pattern usually indicates that the sample is biased, because areas outside of the trapping grid, where the long-distance movers were to be found, were not sampled. Closure violations can produce substantial errors in density estimates, although the impacts of closure violations are neither fully understood nor predictable. For example, in mark-recapture capture histories of Brown Treesnakes simulated over a 16-day sampling period, daily emigration rates of 2 to 4 percent (frequent in the study species) produced errors in population size estimates of 25 to 65 percent (GHR and K. Dean-Bradley, unpub. data).

5. Use the robust model whenever appropriate, as the robust model incorporates the best of open and closed models in a convincing manner.
6. Enumerate the assumptions inherent in the methods of capture/recapture and data analysis. Means, maximum likelihoods, and confidence limits are only as good as the assumptions that went into their computation and provide a context for the results.
7. Categorize and quantify heterogeneity whenever possible. Some analyses include a mechanism for adjusting for latent individual heterogeneity, but such methods are open to criticism. Categorization of sources of heterogeneity adds insight and greatly improves the precision and trustworthiness of estimates.
8. Be sensitive to methodological factors that directly affect a parameter of primary interest. If the focus of a study is population size, for example, investigators should be especially sensitive to factors influencing capture probability. If the focus is survival, he or she should avoid any action (e.g., injurious or disruptive marking techniques) that causes individuals to flee the study area, being particularly alert to time-delayed responses (marked animals do not flee immediately but depart later). Subtle changes in technique during the course of a study may introduce temporal heterogeneity that is distracting at best and may cause appreciable bias.
9. Minimize the number of parameters in the model as much as is biologically reasonable (i.e., eliminate unnecessary parameters), recalling Einstein's famous quip to make everything as simple as possible, but no simpler! Simpler models (use AIC or one of its improved variants, such as QAIC_c, for model selection) are less prone to errors of model design and interpretation. Focus on simple explanatory functions rather than models with different parameters for every occasion: one explanatory factor instead of two; linear rather than quadratic; covariation between two parameters without an interaction; parallel covariation without an intercept parameter rather than covariation with an intercept; equality rather than parallel covariation; fixed values rather than fixed relationships. Recognize and attempt to correct poor model fits revealed by goodness-of-fit tests, but put little faith in failures to reject goodness of fit, especially if data are sparse. Use biological information to break ties in AIC values, or model average if a clear basis for discriminating among equally plausible models cannot be identified.
10. Maximize the amount of information taken from the field and from the data, to minimize bias and maximize precision. When offered a cost-free opportunity to record either presence/absence or counts, record the more information-rich counts. Even if only the information-poor presences are analyzed, later retrospective analysis may be able to extract greater insight from the additional information. If a choice exists, opt for the most information-rich analysis. The optimum mix is obtained with a good model fit using the fewest parameters. Important phenomena that are not modeled often distort the remaining estimates. Sparse data will always appear to justify a simple model, but simple models are not necessarily biologically real or even adequate if based on small data sets. A chosen model is a working hypothesis, not a final answer. It is permissible to build biological phenomena that are demonstrably true based on larger data sets into a model of a sparse data set. This may cause a specific model to be overparameterized under AIC, but it may be biologically justifiable.
11. Use mathematical simulation (simulation algorithms in MARK) to explore the influence of assumption violations and alternative sampling plans on population estimates. For example, to determine the degree to which a small amount of immigration (or other factor) would distort the abundance estimates obtained from a removal/depletion sample, simulate such immigration with the capture probabilities, number of occasions, and other values of the system.

Dynamic Demography

Population Trends over Time

Perhaps the question most commonly asked about a population is, "What is the population's status: stable, increasing, or decreasing?" For native species, the concern is generally whether or not the population (and species) is declining (a one-tailed question). For an introduced species, the question is generally whether or not the population is increasing (also a one-tailed question). Inspection of population density estimates through time will generally indicate the direction of a trend, if present, but it will not indicate whether the trend has occurred by chance. Small changes in abundance will produce a quasi-stable population trajectory. Large changes in abundance over time will appear as a steep curve. A zero slope implies no long-term trend; the population is stable. Thus, the angle of the slope is the important feature, and it is important to know the statistical uncertainty associated with the best slope estimate. Often the key question is whether the confidence limits on the slope estimate include zero. If not, it is reasonably certain that the population is changing. If they include zero, but just barely, we can deduce that the most likely scenario is of change; constancy is also possible, but less probable. If the confidence limits broadly span zero, either the population is stable or the density measurements are insufficiently precise.

Change occurs for a variety of reasons, and information about those reasons is often more valuable than a simple assessment of whether the numbers are going up or down. For example, suppose that the population of an unexploited turtle (i.e., not collected for pets or food) is stable within suitable habitat, but that the amount of suitable habitat is declining, and therefore, the total population is declining. In that case, one would know how to reverse the overall population decline: restore or replace suitable habitat. Conversely, if an unexploited turtle were declining in all habitats, one would not be able to identify a reason without further study.

THE SHAPE OF CHANGE

By stratifying a sample by habitat type or other feature that may identify the cause of a population change, an investigator can increase his or her ability to make inferences about the cause(s) of a decline (or increase). The rate and shape of the population decline (whether the population change is exponential or linear) can also help identify appropriate conservation measures. Consider a scenario in which change in a turtle population is concomitant with change in the amount of a particular habitat type. Is the rate of habitat change constant (i.e., linear), or proportional (i.e., exponential), or does it follow some other pattern? Suppose that from 1990 to1994 there were 10,000 ha of the focal habitat type. In 1994 a new tax law provision made liquidation of this habitat type profitable. Assume that the coverage of the habitat type decreased linearly by 1,000 ha per year each year after 1994 (i.e., to 9,000 ha, 8,000, 7,000,etc.). This model of habitat loss implies zero slope (no loss) from 1990 to 1994, with a linear decline of 1,000 ha/y thereafter. Such habitat change outlines a specific trajectory of expected population change, allowing for a powerful test of causation.

An alternate hypothesis is that fire ants (*Solenopsis*) invaded nests of this turtle, killing hatchlings and causing nearly total loss of recruitment. Perhaps fire ants expanded into the study area in 1994. In that case, one would expect an exponential decay in the turtle population estimates, because a constant proportion of surviving turtle individuals would die of natural causes each year and not be replaced by recruitment. Suppose that 10 percent of the population died each year (i.e., constant proportional change). In that case, one would expect the population trajectory from 1994 to be 10,000, 9,000, 8,100, 7,290, 6,561, and so forth. Naturally, one's population estimates won't follow such a pattern exactly, but the most parsimonious model for the data may be an exponential decay.

Conventional statistics packages will readily fit an exponential curve to such data. One can obtain the same curve by fitting a linear regression to the logs of the population estimates. Using logs instead of raw numbers reduces the size of the variance and tends to equalize variances among the points, which means that data can be properly analyzed with a conventional linear regression.

The alternate hypotheses we have considered up to this point are population constancy through 1994, followed by a linear decline (model A), and population constancy through 1994, followed by an exponential decline (model B). A third alternative would be population constancy throughout (model C). Using conventional least-squares regression models, one can compare the fit of these three models to the data on turtle population size using AIC or one of its variants (AIC values for regression are available from many statistical packages). However, AIC values can be compared only among models that use the same input data. Thus, in our case, one could not compare the AIC value of a linear model in relation to log-transformed input data with that of another linear model based on untransformed data. Using AIC one could compare a nonlinear fit to untransformed data with a linear fit to untransformed data.

Suppose that monitoring data were obtained simply for surveillance purposes (to detect declines from any cause), without any *a priori* reason to anticipate any particular population change. If the data had been obtained using a robust model analyzed with program MARK, one could model lambda (i.e., the ratio of the population size at one time over the population size at the preceding time) as a constant value, a log-linear (exponential), or a linear one. These competing analytical models could be compared in MARK using AIC. In the event that a linear model was the best fit, and the constant model was more than 10 AIC units larger than the linear model, one could conclude that change is most likely occurring. Suppose, however, that the constant model was only 3 AIC units larger. In that case, one might use model averaging to obtain the best estimate of lambda, recognizing that both constancy and change are plausible (although change is more likely).

REGRESSION AND RANDOMIZATION APPROACHES

If population estimates are not collected with a robust mark-recapture model, one can evaluate time-based trends with conventional regression packages, as described in the penultimate paragraph above. Unfortunately, conventional linear regression makes assumptions about the independence, normal distribution of errors, equality of variance, and skew of the input data. Less demanding alternative analyses use randomization or nonparametric methods.

For the randomization approach, one metaphorically deposits the population estimates in a statistical pool from which each combination of points is drawn at random without replacement. Using conventional regression, one computes the slopes of the resulting population estimate strings. From the distribution of all possible regression values, one tabulates the fraction of the sample for which a slope was as extreme as that which was empirically observed. Suppose, for example, that one has five population estimates. For five estimates, 5! (= 5×4×3×2×1), or 120, different orders are possible. Suppose that only two of those have a slope as extreme as the one observed. In that case, the probability of obtaining so extreme a slope by chance is about 2/120, or 0.017, and one infers that the observed change in population density is unlikely to have happened by chance.

The randomization approach is about as powerful as the conventional parametric approach for well-behaved data and sample sizes of seven or more population estimates (Thompson et al. 1998); for data that do not meet the conventional parametric assumptions, it may be more powerful. For smaller sample sizes, the small number of possible permutations limits the power of the test. For example, with only three population estimates, only six permutations are possible; therefore, the smallest possible probability value is 0.17.

MANN-KENDALL NONPARAMETRIC TEST FOR TREND

The Mann-Kendall nonparametric test can be done by hand. It requires only ordinal density estimates (ranks); an exact value for each density estimate is not necessary as long as one knows whether the density was higher or lower in each pairwise comparison of the samples. For simplicity, consider four annual population estimates. The first step is to make pairwise comparisons of years: 1-2, 1-3, 1-4, 2-3, 2-4, and 3-4. Ties are ignored. The number of pairwise comparisons in which the earlier estimate is larger than the later estimate is subtracted from the number of pairwise comparisons in which the converse is true. The test statistic is the absolute value of the difference in counts. Suppose that among the six pairwise comparisons of the four annual population estimates, there are four examples of decline, one example of an increase, and one tie. In that case, the test statistic, *S,* is 3 (four declines minus one increase). The probability of so extreme a value of *S* is read from Table 32, *n* indicating the number of population estimates. Note that one would get the same value of *S* if there had been four increases and one decline. The test is one-tailed and is not concerned with the direction of the population change.

In this particular case, we see that no value is given for the exact test statistic $S=3$ and $n=4$ population estimates. However, we can interpolate approximately between the probabilities given for $S=2$ ($P=0.375$) and $S=4$ ($P=0.167$). Our probability is about 0.27, indicating little basis for concluding that the population has changed. For a sample of only four population estimates, all six of the pairwise comparisons need to be in the same direction (all later > earlier or vice versa) for statistical significance. Had they been so, our *S* would have been 6, with the associated probability of 0.042. One can see from the example that it will be difficult to detect a trend nonparametrically if the sample size of population estimates is small.

Life Tables

A life table is a specific matrix (table) of vital rates: survival, growth, maturation, and fecundity. Life tables are used to analyze demographic patterns as a function of an individual's age or life stage. Instead of being content knowing simply whether the population overall is increasing or decreasing, life tables explore the demographic components that produce population change. Theoretical life tables are often prepared to examine population dynamics conceptually, but in this book we will limit ourselves to the preparation of life tables with data collected in the field from real populations. Obtaining the requisite data is a monumental task, rarely attempted and even less often successfully completed. Indeed,

TABLE 32

Probabilities for the Mann-Kendall Nonparametric Test for Trend[a]

S[b]	Value of *n*				S	Value of *n*		
	4	5	8	9		6	7	10
0	0.625	0.592	0.548	0.540	1	0.500	0.500	0.500
2	0.375	0.408	0.452	0.460	3	0.360	0.386	0.431
4	0.167	0.242	0.360	0.381	5	0.235	0.281	0.364
6	0.042	0.117	0.274	0.306	7	0.136	0.191	0.300
8		0.042	0.199	0.238	9	0.068	0.119	0.242
10		0.0083	0.138	0.179	11	0.028	0.068	0.190
12			0.089	0.130	13	0.0083	0.035	0.146
14			0.054	0.090	15	0.0014	0.015	0.108
16			0.031	0.060	17		0.0054	0.078
18			0.016	0.038	19		0.0014	0.054
20			0.0071	0.022	21		<0.001	0.036
22			0.0028	0.012	23			0.023
24			<0.001	0.0063	25			0.014
26			<0.001	0.0029	27			0.0083
28			<0.001	0.0012	29			0.0046
30				<0.001	31			0.0023
32				<0.001	33			0.0011
34				<0.001	35			<0.001
36				<0.001	37			<0.001

a. From Thompson et al. 1998.

b. *S* = test statistic.

there is no such thing as a final life table, as fecundity and survival values change with time, and one can always add parameters to make the table more complex and realistic.

Why would anyone undertake such a Sisyphean task? Most life tables are generated by investigators engaged in pure research, intent on unraveling the conceptual mechanisms of population biology. Those engaged in applied research may compile life tables to understand the effects of a particular management action. For example, one can determine if harvesting old-growth timber stands reduces the survival of juvenile snakes with life table data from both treatment and control plots. One could measure juvenile survival only, omitting the other values in the life table. Inclusion of those other values makes it possible, however, to determine if an observed change in juvenile survival is likely to result in a population decline or if other mechanisms are compensating for that decline (e.g., through elevated hatchling survival), or if a concomitant population decline has occurred for other reasons (e.g., a reduction in adult survival). Recall the example in the introductory dialogue (see “Chapter Organization,” above), in which Haldre sought life table data to compare the population level consequences of road kills versus collecting animals for pets.

Unequivocally determining the effects of management actions on a population requires an experiment, but a nonexperimental life table can reveal the prevailing demographic conditions. Suppose, for example, that a population of tortoises is declining on one island, but not on another. Life table information from both islands might expose recruitment failure in the declining population, which would allow managers to focus on determining the causes of that recruitment failure. Experimental manipulation of the putative cause of the recruitment failure would be needed to confirm causation, but the life table information would have identified a target early on.

Nonexperimental life tables also provide an opportunity to examine demographic features that may suggest future problems for a population. Consider a long-lived and slowly maturing species, such as a sea turtle, that breeds synchronously at highly irregularly intervals. The number of turtles on the beach during a breeding season may seem to fluctuate erratically. Thus, it may be difficult to assess population trends from counts of nesting turtles. Furthermore, if turtle recruitment is failing, it might be decades before the decline is noticed among nesting females (the population stratum that is usually the sole basis for monitoring overall sea turtle population trends). Compiling empirical data for a life table of a long-lived species may provide insight into problems that would not otherwise be evident for decades.

Unfortunately, some nonexperimental uses of a life table are rarely possible, because life tables are rarely available for intact populations. Typically, interest in preparing a life table appears only after a species is teetering on the brink of extinction and a life table is needed for preparation of a population viability analysis (a special use of life tables, see “Population Viability Analysis,” below). Because adequate demographic data are rarely extractable from an already endangered population, and baseline (pre-decline) population data are rarely available, population viability analyses of most endangered species are of limited utility (or even useless). This situation underscores the value of preparing life tables for species while they are still common and their populations are stable. Conservation biologists need baseline values with which to compare data collected later, when a species may be in trouble. In addition, common species can serve as model systems for understanding the ecology and population biology of reptiles of special interest, including those rare species for which usable data may never be obtained.

BASIC TYPES OF LIFE TABLES: AGE-BASED MATRICES

In life tables, an organism’s life cycle is divided into categories by age class or developmental stage. For each population the number of individuals in each class or stage is estimated, along with the average fecundity and average survival of organisms in that (st)age, and the average growth rate of individuals from each (st)age into the next (st)age. It is perhaps easiest to envision in terms of an age-based life cycle of female humans. For simplicity, let us consider each decade of human life as an age class, and let us assume that the decadal survival of each age class is 90 percent. Suppose that 100 girl babies were born each decade in the recent past. Thus, in this simple case there would be 100 females in the 0–9 age class, 90 in the 10–19 age class, 81 in the 20–29 age class, and so forth. Let us also assume that each living female age 10–49 produces an average of 0.5 female babies per decade, and that no one lives past the age of 79.

Age-based life tables are conventionally portrayed in *Leslie matrices,* named after Patrick Leslie, an early developer of this technique. He proposed displaying the requisite information in a square matrix, which has the same number of rows and columns as there are age categories, in our case eight: 0–9, 10–19 . . . 70–79. The top row of each matrix gives the fertility of each age class. Although the birth rate per female is constant in our example, a female must survive through a period to reproduce; thus, fertility must be discounted to the extent of mortality. Fertility for a given age class is the birth rate multiplied by the survival rate. As no young are produced by girls less than 10 years of age, the first fertility in our example would be zero, followed by $0.9 \times 0.5 = 0.45$, for each of the four fertile age classes. The last three age classes are post-reproductive, and therefore have a fertility of zero. The only other element in the matrix is the age-specific survival rate, which in the Leslie matrix is placed on the subdiagonal (one cell below the matrix diagonal). Our age-based matrix is shown in Table 33.

A life table is formatted so that each column and row in the matrix represents one age class, and the value in each cell represents the *transition probability,* or the probability that an individual in that age class will enter the next age class. Thus, the cell in the second row of the first column represents the probability of an individual passing from the first age class (0–9) to the second age class (10–19). The value in the second row of the second column is zero, because there is zero probability that a teenager will stop aging. Over a decade, each teenager will either survive to become a twenty-something or die. For similar reasons, all of the diagonal elements in an age-based matrix are zero. Naturally, a teenager can transition only to the next older class; one cannot directly become a forty-something, nor reverse the aging process.

You may notice that there is no cell representing the probability of an individual surviving from the eighth age class, as the structure of the matrix assumes a probability of zero for “graduating” from the last age category. Now consider the top row: the value in the second column of the first row represents that probability of an individual in the second age class (10–19) becoming a member of the first age class (0–9). Of course,

TABLE 33

Age-Based Life Table Portrayed in a Leslie Matrix

Age Class	*0–9*	*10–19*	*20–29*	*30–39*	*40–49*	*50–59*	*60–69*	*70–79*
0–9	0	0.45	0.45	0.45	0.45	0	0	0
10–19	0.9	0	0	0	0	0	0	0
20–29	0	0.9	0	0	0	0	0	0
30–39	0	0	0.9	0	0	0	0	0
40–49	0	0	0	0.9	0	0	0	0
50–59	0	0	0	0	0.9	0	0	0
60–69	0	0	0	0	0	0.9	0	0
70–79	0	0	0	0	0	0	0.9	0

a teenager cannot bodily revert in age; they can, however, give birth to a new individual of the younger age class. The number in that cell, fertility, is the probability that a female will do so. Because fertility can result only in new individuals of age zero, fertility values appear only in the first row.

The reason for using this exact format for an age-based matrix is that under the rules of matrix algebra, the product of this matrix and a column vector representing the number of individuals in each age class (an age pyramid) yields a new column vector that is the expected number in each age class at the next time step. In our example, we have used a decade as the time step, but any time interval could be used, as long as all values in the matrix represent transition probabilities of exactly that length of time. The matrix not only produces a new age pyramid but also has several other mathematical properties that produce bliss in mathematicians. For example, the ratio between abundance in the next time step and abundance in the present one is a mathematical property of the matrix called the *dominant eigenvalue*. It is represented by the Greek letter lambda, which is exactly the same lambda that was introduced in the analysis of mark-recapture data. A lambda greater than 1.0 reflects a population that is expanding; a lambda less than 1.0 indicates a population that is declining. The population growth rate, known to population biologists as "*r*" is 1 – lambda. Finally, the matrix has another mathematical property, called the *eigenvector,* or stable age distribution, which is the distribution of ages that will replicate itself exactly. For any given age-based matrix, only one vector (distribution) of ages (the eigenvector) will produce exactly the same distribution of ages in the following time step. It is no wonder that analysts prefer that age-based life history information be formatted as a Leslie matrix.

STAGE-BASED MATRICES

An alternate format for life history information is the stage-based or *Lefkovitch matrix,* named after its originator. The format of a Lefkovitch matrix is the same as an age-based matrix: each row and column represents a class, and the cell contents represent the transition probabilities among classes. In a stage-based matrix, however, classes represent life stages rather than ages. Because an individual can remain in the same life stage for multiple time steps (perhaps up to several years), a stage-based model can have nonzero values in the cells on the diagonal. Furthermore, the time step of a stage-based matrix is more arbitrary. That is, any time interval can be used, but it must be exactly the same time interval for all transition probabilities in a single matrix. For example, the time step of a juvenile stage must be the same length as the time step for the adult stage. One accommodates life stages of different lengths by placing appropriate values in the diagonal cells, allowing an individual to remain in the same stage for multiple time steps.

With stages of different durations, the computation of transition probabilities can become a bit confusing, but stage-based models are the most common form of life history analysis, for at least two reasons germane to reptile studies. One is that many reptiles mature as a function of size rather than as a function of age. Therefore, stages defined by size rather than age may more accurately reflect the biology of the organism. Second, the age of a reptile is generally unknown and cannot easily be determined, whereas its stage often can. In such cases, it may be easier to produce a stage-based matrix. The appropriate justification, however, is that the life history of the focal organism is structured more by stage than by time.

Although knowing absolute ages is not required for a stage-based matrix, an investigator must know the transition probabilities, which requires either longitudinal studies or a method to obtain absolute ages and past growth trajectories. That is, individuals must be monitored long enough to ensure that the fraction of each stage class that makes a transition to the next class during each time step can be accurately estimated.

For a better understanding of transition probabilities, it is useful to think about how an age-based matrix could be converted into a stage-based one. Consider the case of a viviparous reptile species that ordinarily matures at age 2. For simplicity, the matrix tracks only females. Female juveniles of the hypothetical species have an average annual survival of 0.8. Let us further suppose a stable age distribution, a pool of 180 juveniles, and a matrix with a time step of 1 year. Thus, at each yearly turn of the time crank, a juvenile can survive, die, or mature. At the beginning of our time step, there are 180 juveniles, 100 in their first year (younger juveniles) and 80 in the second year (older juveniles, age 1–2 y). Eighty percent of all juveniles survive to the following year (producing

64 mature animals and 80 older juveniles). Thus, the transition probabilities for the 180 total juveniles are that 0.444 (= 80 of 180) survive and remain in the juvenile class, and 0.356 (= 64 of 180) transition to the next stage.

To complete the example, suppose that annual adult survival rate for the species is 0.9, and reproductive senescence does not occur. There is, however, the matter of reproduction. If the species is viviparous, and no other life stages are possible, one could model the species with perhaps just two life stages: juvenile and adult. If the mean number of female offspring produced per adult female is 1.6, the average annual fertility value will be $0.9\times1.6=1.44$, in which case our stage-based matrix will be as shown in Table 34.

TABLE 34

Stage-Based Life Table, or Lefkovitch Matrix

Stage Class	*Juvenile*	*Adult*
Juvenile	0.444	1.440
Adult	0.356	0.900

In this matrix, the first row or column is the juvenile life stage, and the second row or column is the adult life stage. During each average year, each average juvenile female has a 0.444 probability of remaining a juvenile, a 0.356 probability of becoming an adult, and therefore a 0.200 probability of dying. Each adult female has a 0.900 probability of remaining an adult and an average annual fertility of 1.440 female offspring.

CONVERSION AMONG THE BASIC TYPES

Many reptile species are especially vulnerable to predators during a period following hatching or birth. If such a situation applies to our hypothetical species, it may not be appropriate to model the entire juvenile period as a single stage. Likewise, if the species is oviparous, it may be desirable to treat the eggs as a separate life stage. Let us suppose that our hypothetical species is oviparous, with an 80 percent hatching (= survival) rate (after accounting for developmental and predation losses) over the 3-month incubation period. We can continue to use the values previously assigned to our hypothetical species, with one exception. As the eggs do not persist for 1 year, it is not appropriate to set the time step for our matrix at 1 year. The maximum length time step we can use is the duration of the egg stage: 3 months (= 0.25 y).

Converting the time step to 0.25 y presents little problem for fertility, only that the average annual fertility (previously set at 1.6 female eggs) needs to be corrected to one-quarter of a year and discounted by quarterly adult survival (for simplicity assume that our hypothetical species breeds uniformly year round). Recall that the mean annual survival of adults was 0.900, implying that the quarterly survival of adults will be $(0.900)^{0.25}=0.974$ (because the only way an adult can survive for a year is to survive each quarter, i.e., $0.974\times0.974\times0.974\times0.974=0.900$). Therefore, average quarterly fertility will be $1.6\times0.25\times0.974=0.390$ female eggs.

Correcting the survival values is a bit more complex. Recall from above that the average juvenile has a 0.444 annual probability of remaining a juvenile, a 0.356 annual probability of becoming an adult, and a 0.200 annual probability of dying. The simplest situation is that of a juvenile remaining a juvenile for another year by surviving for four quarters in a row. What probability raised to the fourth power yields a 0.444 annual probability of remaining a juvenile (i.e., $x^4=0.444$)? The answer is 0.816 (= $(0.444)^{0.25}$). Similarly, a juvenile that becomes an adult during a year can do so only by becoming an adult in one of the four quarters and surviving as an adult through the subsequent quarters. We now have the information (Table 35) we need to compute the quarterly probability of a juvenile becoming an adult and surviving the year.

We know that the annual probability of becoming an adult and surviving is 0.356, and from the above table we can solve for *x*, the quarterly probability of becoming an adult, because it is the sum of the alternate possibilities, mathematically expressed in the last column of the table ($0.924x+0.774x+0.649x+0.543x=2.890x=0.356$, or $x=0.123$).

Therefore, our three-stage matrix, adapted to a quarterly time step would be as shown in Table 36. Notice that all of the values have changed from the preceding matrix. The size of the time step is very important! We see that the average female fertility is 0.390 female eggs per quarter, and that these have a 0.800 probability of surviving during the quarter to hatch (stage 1 to stage 2). Most (0.816) of the juveniles remain juveniles (stage 2 to stage 2) during a quarter, but a

TABLE 35

Sample Data for Calculating Quarterly Probabilities

1st Quarter	*2nd Quarter*	*3rd Quarter*	*4th Quarter*	*Expression*	*Value*
Becomes adult (x)	Adult survival (0.974)	Adult survival (0.974)	Adult survival (0.974)	$(0.974)^3\times x$	$0.924x$
Remains juvenile (0.816)	Becomes adult (x)	Adult survival (0.974)	Adult survival (0.974)	$(0.974)^2\times(0.816)\times x$	$0.774x$
Remains juvenile (0.816)	Remains juvenile (0.816)	Becomes adult (x)	Adult survival (0.974)	$(0.974)\times(0.816)^2\times x$	$0.649x$
Remains juvenile (0.816)	Remains juvenile (0.816)	Remains juvenile (0.816)	Becomes adult (x)	$(0.816)^3\times x$	$0.543x$

TABLE 36

A Three-Stage Matrix Adapted to a Quarterly Time Step[a]

Stage Class	*Eggs*	*Juvenile*	*Adult*
Eggs	0	0	0.390
Juvenile	0.800	0.816	0
Adult	0	0.123	0.974

a. Data from Table 35.

few (0.123) mature (stage 2 to stage 3). Adult survival (stage 3 to stage 3) is fairly high, 0.974/quarter.

ANALYSIS OF LIFE TABLES

Would a population with vital rates such as these sustain itself? To determine this we need to compute lambda, the dominant eigenvalue. If lambda is greater than 1, the population will sustain itself after it reaches a stable stage distribution (although it may decline until it reaches the stable distribution). The easiest way to compute lambda is to plug the above matrix into one of the many computer packages that is tailored to life-table analysis. RAMAS software makes several products that are suitable—RAMAS/Ecolab, RAMAS/GIS, RAMAS/Metapop, RAMAS/Stage—and there are several other reputable products (ALEX and VORTEX), some of which are available free through websites (see Appendix II). Many of these products are intended primarily for population-viability analysis, a specialized form of life table analysis. They are complex, and most have highly sophisticated algorithms for assessing population trajectories under a wide variety of conditions (see "Population Viability Analysis," below). Careful evaluation of program performance and function is a prerequisite to proper use of these tools.

Returning to the question at the beginning of the preceding paragraph, the lambda associated with the three-stage life table is 1.098, indicating a rapidly expanding population (increasing nearly 10% per quarter). If lambda were to hold constant, the population would expand more than six-fold in 5 years. Obviously, such an increase could not be sustained for any appreciable time interval, because the values of the matrix would begin to change in response to changing conditions associated with a much denser population. Recruitment could decline, or predators might key in on the rich food source and increase mortality. It is important to remember that lambda is projected on the basis of current conditions; it is not a prediction of what will occur in the future. To make a realistic prediction, one would need additional information about stochastic events, catastrophes, genetic makeup of the population, density dependent population parameters, and so forth. Some of these are considered briefly below, but first let us turn to the problem of obtaining the field measurements needed to prepare a realistic life table.

DATA NEEDS FOR VITAL RATE ESTIMATION

For each life stage or age class one needs the following stage- or age-specific data per time step: (1) average probability of survival, (2) average probability of progressing to the next st(age), (= transition probability), (3) average fecundity, and (4) any time-based limits on these values (e.g., reproductive senescence or maximum longevity). These values are termed *vital rates*. For the model to be realistic, we also need to discover the form of density dependence. Note the use of "average" as the measure of central tendency in the above list; in some cases, an alternate measure may be needed to characterize highly skewed distributions.

SURVIVAL

Traditionally, the fate of an individual that disappears from a sample is determined with radiotelemetry, which is an excellent tool for the individuals that are large enough to carry a transmitter without reduction in their survival probability. Unfortunately, most reptile species are too small. In addition, radios must have a lifespan long enough to provide a reasonably precise estimate of survival. If, for example, an annual survival rate is in the neighborhood of 0.95, only 1 of 20 animals is expected to die naturally over the course of a year. In this case, one would need a very large sample of animals with very long-lasting radios to estimate the annual value with enough precision to produce a usable life table.

Mark-recapture is another means of estimating survival. Recall that open models estimate apparent survival, which combines losses due to death and emigration. To obtain a survival rate one must tease apart these two causes of animal disappearance. The problem is especially acute for dispersing life stages, in which survival is often lowest and emigration highest. Isolated populations are often studied because their dispersal is naturally limited. Unfortunately, such populations may have unnatural survival values because of their isolation, or they may be source or sink populations, with correspondingly unrepresentative life tables. If one can sample two or more sites that receive dispersing individuals, it may be possible to use a multi-stratum mark-recapture model to assess the rate of dispersal between strata. An intensive mark-recapture effort sometimes allows one to census the individuals born into a population and to infer that later arrivals have dispersed from adjacent sites. However, investigators must monitor an area large enough to ensure that long-distance movers will be detected. Most do not.

Investigators must be alert to the possibility that some aspect of their monitoring technique may influence the phenomenon of interest. For example, marking techniques that reduce survival even slightly can seriously bias long-term survival estimates. Factors that influence detectability, such as water level (crocodilians) or occasional rain storms (desert species), can bias estimates of apparent survival through covariance between detectability and apparent survival.

If the purpose of life table preparation is to conduct a population viability analysis, one will need to determine the stability of survival and density values over time. Measurements of these vital rates incorporate both process variation (real change in the parameter of interest) and sampling variation (measurement error). To segregate these two sources of variability, it is essential to have multiple measurements of the traits during static conditions so that sampling variation can be estimated. Rare events, such as hurricanes, complicate the estimation of process variance: "Accurate estimates of variance in vital rates probably require at least 1–2 generations of study, which could easily exceed 10–20 years for long-lived vertebrates" (p. 830 in Beissinger and Westphal 1998, a key critique of life table analyses). Pimm and Redfearn (1988) found that variance in insect census data stabilized after about 8 years, but that stability in vital rates for birds and mammals required 30 to 40 years of data.

MARK facilitates the independent estimation of process and sampling variation, if the required replicate samples are available. MARK can also estimate survival values from radiotelemetric ("known fates") monitoring.

GROWTH

If a study species can be aged through a "snapshot" technique such as skeletochronology (which may give an individual's absolute age at the time the bone was sectioned), it may be possible to develop reasonable growth rate estimates from the distribution of sizes versus ages. This approach may require the assumption that cohort sizes have been stable over time and that survival and growth are not correlated (e.g., if fast growers disperse from the study site or die, the growth of the remainder—which gets sampled—may not be representative of the population). If one can estimate the previous size of individuals from the deposition of bone between annuli, it may be possible to estimate growth for surviving individuals independent of past fluctuations in cohort size. Failure of the required assumptions can produce significant bias in growth estimates. Direct measurement of growth is preferable, as long as the probability of dispersal away from the study site is not correlated with growth (fast- or slow-growers more likely to disperse?). Growth measurements of captive animals are not a reasonable substitute for measurements of growth in the wild. Growth rates of captives may differ radically from those of wild individuals if the latter are food-limited or if captive individuals are stunted from the lack of some unrecognized herpetocultural requirement.

FECUNDITY

Direct measurements of reproduction are ideal. However, encountering parturient or ovipositing females just as they are giving birth or laying eggs is unlikely. Most researchers estimate fecundity from samples of follicles, fetuses, egg masses, or litters, and knowledge of the fraction of the female population that reproduces during any particular reproduction opportunity. The robust mark-recapture model can sometimes be used to identify the fraction of the female population that is either present at an oviposition site during each breeding season, or absent from a nonbreeding home range (and returning thereafter). Such measurements of fecundity should be fairly accurate if one recognizes that the number of young born alive or the number of viable eggs laid is less, sometimes substantially less, than the number of fetuses or follicles that reach full term. In many species, fecundity is related to female size, which can be an important modulator of reproductive output particularly among populations whose size or age distributions differ. Dunham and colleagues (1988) provided a good overview of the challenges of estimating recruitment from field measurements.

LONGEVITY AND SENESCENCE

Most reptiles die before becoming post-reproductive because of old age. For many reptile species, reproductive senescence, if it occurs at all, is unlikely to be of demographic significance. Reproductive senescence can be detectable in special groups (e.g., island forms, populations after a steep population decline), however. Senescence can markedly influence estimates of lambda based on life tables. If an investigator suspects senescence, he or she should simulate a realistic life table matrix to determine if the potential influence is large enough to be important. If so, it would appear desirable to account for reduced output by stratifying fecundity estimates by age or size.

FORM OF DENSITY DEPENDENCE

Density dependence is among the most problematic aspects of life table preparation. One cannot usually determine the mechanism or form of density dependence from observational studies, yet analytical models that lack a density dependence component are clearly meaningless for projection beyond a few years (densities grow or decline exponentially). Experimental manipulation may be required to force an ecological system into a condition in which density compensation appears or becomes measurable. For example, one might remove every other animal from a population to see what effect this has on recruitment and survival. Augmentation is less likely to be successful, because it is apt to induce rapid emigration. Such experiments may be prohibitively expensive, but without information on density dependence, it may be unrealistic to use a life table for management planning or evaluation. Because density dependence is so difficult to measure, many of the life table software packages provide hypothetical forms of density dependence of varying magnitude that can be plugged into a model to curb runaway population declines or increases. Whether these hypothetical values are reasonable substitutes for empirical information in a realistic life table analysis is arguable.

LIFE TABLE MODEL VALIDATION

Few data sets are large enough to permit validation of a life table from available data. Ideally, one would compile the empirical data needed for each cell of the life table and then incorporate model mechanisms to account for the complexities of the real world. Typically, the exact form of these mechanisms is estimated from model behavior, with inputs and mechanisms tweaked to provide population size estimates that match known values. Once those adjustments have been made, the resultant model is tested against population estimates that were not used in the model construction. A good approach is to split a long data set into first and second halves, generate and fine-tune a model with the first half of the data, and then test its predictions against the observed behavior of population data collected subsequently. This approach has produced mixed results the few times it has been attempted (e.g., Brook et al. 1997, 2000). With excellent input data sets (Brook et al. 2000), the results have been gratifying; with data of more typical quality, results have been poor (Brook et al. 1997).

The models that have been tested vary in their sophistication. A basic *deterministic model* estimates population trajectories from mean values for each stage. Realistic year-to-year variation is added to the vital rates of each cohort to create a *stochastic model*. *Individual models* incorporate random variation in individual performance in addition to the variation added to the vital rates of the cohort. Spatially explicit models also incorporate geographic variation in either individual or cohort vital rates. Models that incorporate natural or artificial selection are called *genetic models* and may have particular value for very small or intensively managed populations, such as species being maintained or augmented by captive propagation. The necessity of adding these features will depend on the attributes of the system being studied. Soulé (1987) and Caswell (2001) are good sources of information for evaluating the importance of these model elements.

Population Viability Analysis and the Use of Realistic Projection

A *population viability analysis* (PVA) is an assessment, based on realistic estimates of vital rates, of the probability that a given population will decline (decrease in size) to extinction or to a critical level (such as 100 individuals) that threatens extinction. Preparation of a realistic PVA is almost always stimulated by concern over a critically endangered species. Typically, these species are so rare that estimating their vital rates accurately is not possible. Consequently, scientific opinion is sharply divided on the merits of attempting a PVA for most species. Many commentators have stressed that "collecting sufficient data . . . is simply not practical in most cases" (Boyce 1992, p.482; also cited in White 2000). Nonetheless, PVAs continue to be generated, and in selected reptile cases, with considerable value. White (2000, p. 324) noted that "PVA can be viewed as a heuristic tool to explore the dynamics of an endangered population, but not as a predictive tool."

Among the many PVAs for vertebrate taxa, the most fruitful have been those created to evaluate sea turtle management. Initial efforts to conserve sea turtles focused on giving baby turtles a head start by holding them in captivity until they reached a size with reduced vulnerability to predators. However, sea turtle PVAs indicated that realistic values for enhancement of survival of juveniles would not be sufficient to prevent extinction (Crouse et al. 1987; Crowder et al. 1994, 1995; Crouse 1999). The PVAs indicated that reduction in adult mortality was needed to reverse population declines in sea turtles, a finding that led directly to the legal requirement for turtle excluder devices (TEDs) on fishing trawls (U.S. National Research Council 1990). This is a classic example of the successful use of a PVA as a "heuristic tool to explore the dynamics of an endangered population" (White 2000, p. 324) when the data were insufficient to allow a realistic population projection.

PVAs should be used to judge the ecological costs of commercial exploitation of many reptile species. For example, one could ask what impact the annual export of 200,000 box turtle juveniles would have on populations in the United States. In most cases, the data will not exist for usable analyses of this sort. Even if estimates could be obtained for each cell of a requisite life table, and the appropriate model determined, it is unlikely that adequate information would be available to assess range-wide density dependence.

The leading software programs (see Appendix II) for PVA include various RAMAS products, VORTEX, and numerous academic programs that seem not to be fully supported (e.g., ALEX).

ALTERNATIVES TO PVAS

One popular alternative to PVAs is *Adaptive Management* (Holling 1978; Walters 1986). This approach involves iteratively perturbing a system by a known amount (e.g., initially allowing export of 20,000 box turtles) and then carefully monitoring it to assess the magnitude and direction of the population response. The perturbation is then adjusted (e.g., the take quota is changed) as necessary, in light of the results, to move the population closer to the management goal (e.g., population stability or increase). One hazard of this approach is that the monitoring may not be sufficiently precise to detect important changes in a timely manner. In addition, perpetual monitoring requires a commitment of resources longer than politicians may be willing to sustain. Economic motives might also lead to interference with a monitoring effort. For example, allowing a take (= perturbation) could lead to the development of a political constituency that would resist a reduction in the take quota even if compelling scientific evidence were to indicate that the perturbation is harmful to the population. Such a constituency could lobby to reduce the funding of monitoring programs to prevent compelling scientific evidence from being collected.

Another alternative to a realistic PVA is the management approach taken under the Marine Mammal Protection Act (see Caswell 2001). To ensure that human-caused mortality is kept below a level that will cause depletion of a population, the act requires a comparison between the potential population growth rate under unexploited conditions and a proposed human-caused mortality rate (i.e., commercial take). Three values that provide a margin of error appropriate for the degree of uncertainty and risk associated with the system are estimated: the population size (the minimum of several estimates is used), the potential population growth rate ($r = \lambda - 1$), and a recovery factor, F ($0.1 < F < 1.0$). Because lambda can be estimated from changes in population size over time, life tables, growth rates, and survival rates are not required.

The estimation of life table values for reptiles has hardly begun. Vital rate estimates exist for less than 0.5 percent of reptile species, and most of the constituent field data have recognizable weaknesses. Evaluation of such data is necessarily tentative, yet critical. Field techniques and mathematical tools are improving rapidly, and the preparation of life tables has become a recognized specialty within the field of population estimation. The field has its own software, literature, and experts. These should be closely monitored for continuing advances in this exciting area.

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CHAPTER SIXTEEN

Studying and Monitoring Exploited Species

LEE A. FITZGERALD

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Challenges Confronting Researchers Studying Exploited Species

It never stops for Haldre. As if negotiating the theory and practice of counting lizards that she confronted in Chapter 15 was not enough, Art called and said, “We need to figure out the impact of the pet trade on our Blue-tongued Mango Skinks [a fictitious species; Hiaasen 1991]. The notoriety they have received because of our conservation work has driven their pet-trade price through the roof. In addition, the number of arrests for poaching on the refuge has gone up, which means that collectors are going to great lengths to find them. Some people say the Blue-tongued Mango Skink will be overexploited to the point of extinction! The Vice-Minister has ordered us to work on this immediately.” One can imagine the swarm of thought bubbles crowding together over Haldre’s head (e.g., “Right. I think you meant to say that you are ordering *me* to work on this immediately.” “I know exactly where this is going. There is nobody else to do this work, which means dropping my studies of the population biology of the skinks. How am I supposed to inform managers about lizard demography in the refuge and address the international trade in amphibians and reptiles at the same time?” After an awkward silence, Art asked, “Haldre, are you still there?” Haldre chuckled, and then managed a professional response, “Although mango skinks have appeared sporadically in the pet trade for 20 years, the species that are popular among herpetoculturalists shift all the time. Blue-tongued Mango Skinks are like most lizards (and other reptiles and amphibians) that are kept by people. They are not protected anywhere outside the refuge, they are not listed in any of our national and provincial policies about non-game wildlife, and none of our legally binding trade-monitoring systems pertains to these animals. Also, several other species in the genus are difficult for the layperson to distinguish from Blue-tongued Mango Skinks. All we can get is very coarse export/import data, many of which do not identify the species.” Art replied with a question that Haldre knew was coming: “What would it take to get better data and answer the question of impact?” Haldre wondered, “Art, would the Vice-Minister be willing to push for a regulation banning the sale of all lizards in the genus of mango skinks to solve this issue?” “No way,” Art scoffed, “. . . too much opposition from the pet lobby and concern from the Division of Conservation that enforcement would be a nightmare. The Vice-Minister’s advisors are also concerned that such a policy would deprive kids from catching critters and growing up to be conservationists. After all, that’s how we got our start, isn’t it?” Not wanting to go down this path, Haldre signed off, “OK Art, I’ve got to go check the lizard trapping grids. I’ll get back to you.”

Although the scenario above about trade in the fictional endangered Blue-tongued Mango Skink is hypothetical, it is not far from reality. Surprisingly large numbers of crocodilians, turtles, lizards, snakes, and frogs are harvested to be sold either live, as pets, or dead for their skins, medicinal uses, and/or food (Luxmoore et al. 1988; Fitzgerald 1989; Ceballos and Fitzgerald 2004; Schlaepfer et al. 2005; Warkentin et al. 2009). Millions of individuals from hundreds of species of amphibians and reptiles are taken every year throughout the world for subsistence and the commercial trade, yet large-scale assessments of the sustainable use of particular amphibian and reptile species are only beginning to emerge (Mieres and Fitzgerald 2006). There is no question that commercial exploitation affects species. Many species of crocodilians and turtles have been severely impacted by hunting over much of their

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geographic ranges (King 1989; vanDijk et al. 2000). Snake, lizard, and frog species have also been impacted, both indirectly (e.g., disease transmission, microhabitat destruction) from collecting, and directly by overharvest (e.g., Gorzula 1996; Fitzgerald and Painter 2000; Webb et al. 2002; Carpenter et al. 2004; Zhou and Jiang 2004; Fisher and Garner 2007). With the exception of some crocodilian species (Bradshaw et al. 2006), modern adaptive management models for exploited amphibians and reptiles have yet to be developed. Not surprisingly, resource managers in many countries share legitimate concerns about increasing wildlife trades and the status of commercially exploited species about which we know almost nothing.

It is a positive sign for herpetological conservation that more governments are shifting from unregulated exploitation to management and sustainable harvest. As this shift occurs, large datasets from monitoring programs will be needed to fuel sustained-yield models and advance our understanding of the ability of populations to sustain take or removal. In this section I review approaches for evaluating the exploitative use of amphibians and reptiles and discuss strategies for implementing long-term monitoring programs. Although subsistence use of species is important, I emphasize commercial use of species because of the magnitude and importance of global trade in these organisms.

As is evident from Chapter 15, quantifying population size and vital rates for almost any species is a tall order even for a single study site. In addition, trade and/or subsistence hunting take place in different habitats over broad geographic areas, complicating the assumptions underlying methods of population estimation even further. Replicating estimates throughout the range of commercially exploited species is simply not feasible (Fitzgerald et al. 1991; Caughley and Sinclair 1994; Shine, Ambariyanto, Harlow, and Mumpuni 1998; Shine, Harlow, Ambariyanto et al. 1998) and would not eliminate the need to monitor the take.

Several authors have suggested that analyzing harvest data might provide valuable information for evaluating population trends of exploited reptiles (Norman 1987; Fitzgerald et al. 1991, 1994a, 1994b; Shine, Ambariyanto et al. 1999b; Fitzgerald and Painter 2000). Harvest monitoring is not new. Biologists have long used data collected from wildlife harvests to evaluate population trends (Getz and Haight 1989), just as fisheries biologists have used catch data (Walters 1986). Although complex harvest models exist for fisheries and some game animals, they are largely lacking for amphibians and reptiles. We are just beginning to determine the numbers of each species that are hunted and sold despite the fact that millions of herps are exploited each year to supply a global trade. This chapter thus serves as a basic entry into developing time-series data sets on exploited species of herps and what such datasets can, and cannot, tell us. It is abundantly clear that much research needs to be done on development of harvest models for exploited herps.

For most amphibians and reptiles that are hunted on a large scale, the species are simply exploited according to market demand; management programs do not exist. In clandestine trades, when no one (neither governments nor local communities) has authority over species use, obtaining access to harvested animals and reliable quantitative information on the harvest can be challenging. Still, much information can be gleaned from market surveys, import/export databases, and interviews with people involved in the trade (Fitzgerald et al. 2004). Once management programs are developed, it will be possible to gather higher quality harvest data efficiently and at relatively low cost (Fitzgerald 1994b).

Biologists require basic life history information on exploited species if they are to monitor their statuses, adequately. Such information, as well as a comprehensive knowledge of trade patterns, can be obtained through the examination of harvested animals. Trade patterns describe how hunters obtain animals, how the animals are traded through middlemen, the number and distribution of markets where they are sold, and international import/export arrangements. Researchers embarking on studies of exploited species routinely establish relationships with hunters and traders who can supply valuable traditional ecological knowledge about the species and information (often quantitative) about their hunting efforts and the histories of their hunting areas, trade patterns, and harvest.

After synthesizing data on trade patterns with the demographic information from harvested animals, biologists can draw inferences about population trends of a species and the effectiveness of conservation and/or management policies. Ultimately, they should be able to make informed decisions about the likelihood that a species can be used sustainably and at what level.

Trade Patterns of Exploited Reptiles

The bulk of reptiles and amphibians that are traded originate from developing countries in the Southern Hemisphere and are exported to developed countries mostly in the Northern Hemisphere where demand exists for skins, pets, and exotic foods (Fitzgerald 1989). The concentration of economically important species in areas occupied by rural and indigenous people combined with international currency differentials has set the stage for establishment of collection networks within which traders purchase animals at low prices in local currency and exported them for their U.S.-dollar value abroad.

Trade systems for exploited reptiles have the same general characteristics as systems for most commercially traded wildlife everywhere in the world. Typically, many casual hunters contribute relatively few animals to the harvest, and a few expert hunters collect large numbers. Most rattlesnake hunters in the United States, for example, collect relatively few snakes, whereas the top two hunters in any given year together usually harvest 25 to 85 percent of the rattlesnakes taken (Fitzgerald and Painter 2000). Similarly, thousands of tegu lizard (*Tupinambis* spp.) hunters in rural Paraguay and northern Argentina contribute a few skins to the total harvest, whereas a few professional hunters each provide hundreds of skins per season (Norman 1987; Fitzgerald 1994a). More than 76 percent of the 16,110 turtles traded commercially in Texas during 1999 were collected by two individuals (Ceballos 2001; Ceballos and Fitzgerald 2004).

The general observation that few hunters contribute most of the harvest, whether in turtles, rattlesnakes, tegu lizards, or other species, has significant implications for monitoring and management. Biologists may be able to collect large amounts of data on a target species by working with the most productive hunters. Additionally, policies that influence professional hunters will have a disproportionate impact on the total harvest. Imposing bag limits, for example, constrains the activities of relatively few hunters but has a profound effect on the size of the total harvest (Fitzgerald and Painter 2000).

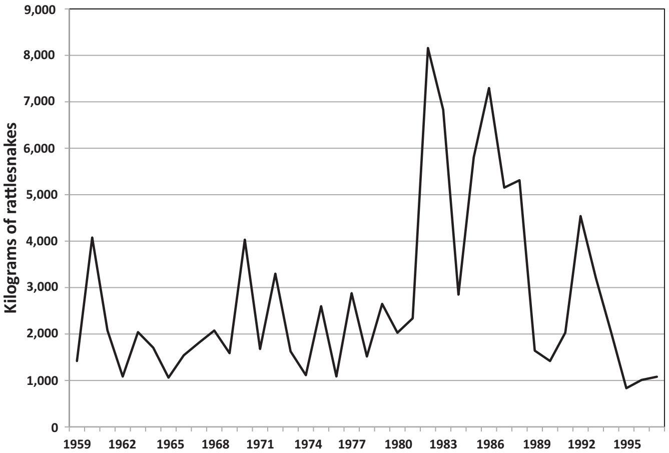

FIGURE 95 Kilograms of rattlesnakes brought to Sweetwater, Texas, rattlesnake roundups, 1959 to 1997. This time series shows that the total weight of rattlesnakes harvested varied among years, with peaks and valleys presumably corresponding to changes (increase or decrease) in amount of hunting in response to changes in market demand. (From Fitzgerald and Painter 2000; © The Wildlife Society, reprinted with permission.)

Hunters generally sell animals or skins to a local buyer who then resells them to a middleman, another level up, and so on, until the harvested animals finally reach a few processing facilities. The number of middlemen in a trade chain depends on the geographic extent of the trade and how well the trade is managed. In general, hunters and several lower levels of middlemen who are engaged in illegal (black-market) wildlife trades in developing countries receive little money. Middlemen tend to drop out of well-managed systems, however, because hunters quickly learn the market value of harvested animals and develop means to access higher (and more just) payments (Fitzgerald 1994b). Regardless of the number of levels in a trade chain, harvested animals accumulate as they are passed up the hierarchy. At processing facilities such as tanneries, abattoirs, or pet warehouses, live animals or their products are finally sold to local distributors or exported.

Natural History and Demographic Information from Hunted Animals

Because collection points in trade chains accumulate large numbers of harvested reptiles, they provide biologists with an opportunity to assemble large datasets on the harvested species, the hunters, the traders, and the trade patterns. Species identification, body size, and sex can often be determined by examination of harvested reptiles, potentially providing large amounts of demographic information. Moreover, collecting data on harvested animals is very cost effective compared to mounting an expedition to collect specimens for study, because the costs of finding and collecting animals are absorbed by the exploitation system.

Various aspects of natural history, such as reproductive biology, diet, and parasite loads, as well as morphological variation and sexual dimorphism, have been described for many species of reptiles based on examination of hunted specimens. Some examples include *Tupinambis* lizards (Fitzgerald et al. 1991, 1993), Common Water Monitors (*Varanus salvator*; Shine et al. 1996), Reticulated Pythons (*Python reticulatus*; Shine, Ambariyanto et al. 1999a), cobras (*Naja sputatrix*; Boeadi et al. 1998), rattlesnakes (*Crotalus* spp.; Fitzgerald and Painter 2000), and sea turtles (e.g., Dermochelyidae and Cheloniidae; Fritts 1981, 1982). These studies provide life history information, such as clutch size and clutch frequency, size at first reproduction, and the relationship between body size and clutch size, which is needed for evaluating prospects for sustainable harvest and for developing population-based conservation strategies. In addition to providing natural history data that can be incorporated into monitoring programs, harvested animals have been catalogued as voucher specimens for natural history collections, and live animals from the pet trade have been used as subjects in many fields of experimental biology.

Harvest Data, Hunter Effort, and Hunting Patterns

Unfortunately, data on the numbers of animals in the trade cannot provide a conclusive answer to the question of sustainability. Harvest data provide an indirect measure of population parameters, but taken alone, they do not reveal the magnitude and causes of population growth rates or the structure of the exploited population. Population structure and growth rate are dependent on qualitative and quantitative characteristics of the animals removed, annual variation in reproduction, geographic variation, population differences among habitats, and other factors that cannot be easily discerned by quantifying the harvest.

A monitoring program may show that the number of animals being harvested is stable, increasing, or declining through time. For example the number of rattlesnakes brought to the Rattlesnake Roundup (a rodeo-like competitive event at which snake hunters capture [and usually destroy] wild members of the genus *Crotalus*; Mushinsky and Savitzky 2006) in Sweetwater, Texas, increased or decreased during some periods (Fig. 95). The world trade in tegu lizard skins has also fluctuated (Fig. 96). The trade in tegu skins from 1977 to 1981 skyrocketed alarmingly, from 1983 to 1987 it showed no trend, and from 1996 to 2005 was lower and relatively stable. In both the rattlesnake and tegu lizard examples, we can see that the data available may not reflect any long-term trend and probably

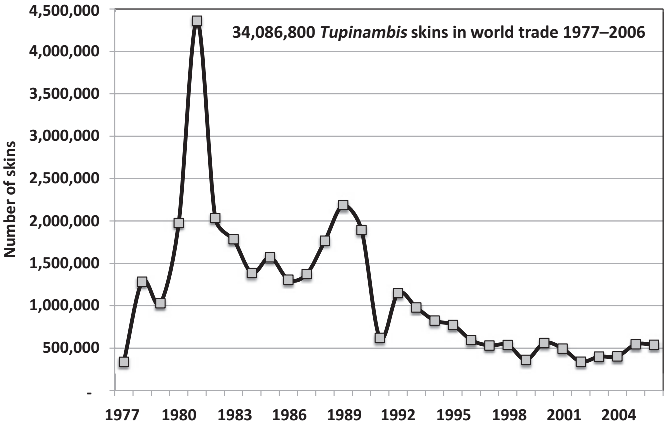

FIGURE 96 World trade in *Tupinambis* lizard skins, 1977 to 2006. Data were obtained from the CITES trade database, maintained by the World Conservation Monitoring Centre. The trade in *Tupinambis* skins peaked when international demand was high and when international currency exchange rates favored the exportation of skins from Argentina to North America, Europe, and Asia. The trade increased markedly from 1977 to 1981 and in 1989; however, the number of skins entering the trade declined after that and was relatively stable from 1995 to 2006.

cannot be relied on to predict what may happen to the harvest in the future. Although graphs of harvest data elicit intuitive feelings about how populations are doing in the face of hunting pressure, each temporal pattern of a rising, falling, or stable harvest can fit a population that is crashing, expanding, or stable.

Incorporating information on the size distribution and sex of animals in the harvest provides a more reliable basis for determining the effects of harvest. A stable harvest of animals accompanied by a significant decline in the average size of the animals through time, for example, is an indicator that hunting may have affected demography of the population. If information is available on how much effort is expended to obtain the harvest, even stronger inferences can be drawn about the effects of hunting.

Hunting effort, strictly defined, is a quantitative term referring to the *time* a hunter needs to bag prey. The average contribution of each hunter to the total harvest is an overall measure of *catch-per-unit-effort* (CPUE), a metric commonly used in fisheries models. The importance of incorporating information on hunter effort into monitoring systems for exploited reptiles can be elucidated by simple examples. If CPUE were constant, one could conclude that a stable harvest over time presumably resulted from a relatively constant rate of removal; there would be no indication of population growth or decline. However, if hunter effort in the system were increasing (e.g., from more hunters working or each hunter working harder for the same catch), yet the number of reptiles harvested remained the same, the CPUE would decline through time, reflecting a scarcity of animals due to a declining population. Increasing effort and decreasing the harvest would be consistent with the idea that the population is in danger of overexploitation. Interpretation of demographic changes in size and sex ratio of harvested animals make inferences more robust.

Conversely, when population numbers are high, hunters may expend less effort to achieve a constant level of harvest, and the CPUE may increase through time. What about the situation in which both the CPUE and the number of animals harvested increase? In this case, a biologist (Haldre's boss, for example) may be alarmed about the prospects for sustainability, but the harvest data do not provide the information needed to evaluate this. The population could be on the verge of collapse from overexploitation, or the population might be expanding and the hunters responding to an abundant resource that is easy to exploit. Clearly, harvest trends through time are best interpreted in light of information on hunter effort, animal demography, and the geographic distribution of hunting.

Unfortunately, these crucial datasets are severely limited for almost all exploited amphibians and reptiles. Long-term time-series data are lacking in most instances, yet short-term data can clearly lead to inappropriate conclusions (Pechmann et al. 1991, Fitzgerald 1994a). Furthermore, data on hunter effort and demography of hunted amphibians and reptiles are lacking. Almost all of us would find ourselves in Haldre's shoes, starting from scratch on an exploitation problem. Haldre needs information on the biology of the Blue-tongued Mango Skink, including knowledge of geographic variation in life history parameters and how hunting, demography, and vulnerability of the skinks vary according to habitat. The trade is illegal and therefore clandestine, so rigorous long-term data on numbers taken and their geographic origins are nonexistent. No one knows how many people are involved in the trade, how much they hunt, and where the harvested animals go. On the brighter side, studies of the exploitation of reptiles and amphibians are accumulating, and we can learn from these cases.

It is clear from data on the world trade in reptile skins, the pet trade, hunts of Western Diamond-backed Rattlesnake (*Crotalus atrox*) over the long term, and turtle trades that hunter effort varies according to market demand, prices, state of the local economy, international currency exchange rates, and conservation policies. Peak harvests of tegu lizards in 1981 and 1988, for example, were clearly due to more hunters working harder when payments to hunters for skins were high because of high foreign demand. This pattern was facilitated by a favorable foreign exchange rate for international skin traders who bought in pesos and sold in dollars. As international demand has waned, so has the harvest (Fig. 96; Fitzgerald 1994b). Thus, at least at the broadest international scale, effects of market demand on hunter effort were apparent. However, lack of detailed information on hunter effort at the local level continues to constrain interpretation of harvest data on *Tupinambis* species (Mieres and Fitzgerald 2006).

Developing methods to measure hunter effort remains the biggest challenge for emerging management programs for exploited reptiles. It is inherently difficult to measure hunter effort in systems where hunters are numerous, uncontrolled, and highly dispersed. Measurement of hunter effort remains a rich area for research on management of hunted amphibians and reptiles. Biologists are increasingly using community-based approaches to work directly with hunters to gather information about hunter effort, hunting methods, and hunting seasons (Fitzgerald 1994b; Fitzgerald and Painter 2000; Noss et al. 2005). Hunters are an excellent source of specimens for natural history studies, because the geographic origin of specimens can be traced. Hunters also provide information on ancillary uses of harvested animals and peripheral trades, such as for meat in the case of skin trades or gall bladders and fat for medicinal purposes.

Interactions between hunters and the species they exploit can be elucidated with monitoring data and have important implications for conservation. Examination of seasonal hunting patterns for tegu lizards in Argentina revealed that more and larger males were hunted during the spring breeding season. Similarly, comparison of hunting patterns for Western Diamond-backed Rattlesnakes among regions showed that differences in sex ratios of the harvested snakes were probably due to den hunting early in the season in some areas, versus road cruising later in the season in other areas. Managers can take advantage of the differential susceptibility of male reptiles during the breeding season to influence the demographic makeup of the harvest by regulating hunting methods and seasons (Fitzgerald et al. 1991; Fitzgerald and Painter 2000). Ecological interactions between hunters and reptiles can only be deduced from a synthesis of data from the harvested animals and data about the hunters. Gaining an understanding of hunting patterns should be a focal point of monitoring programs for exploited reptiles.

Analysis and Interpretation of Exploitation Data

Datasets obtained from each level of a trade network have particular applications and limitations (Box 10). Data on the geographic origins of specimens and dates of harvest obtained from individual hunters can be used to analyze geographic variation of the harvested species and geographic variation in hunting patterns. At this scale the exploitation data can then be used to evaluate policies, such as individual bag limits, aimed at hunters. However, small sample sizes often preclude one from inferring harvest patterns over large areas or evaluating the effectiveness of policies aimed at the total harvest and trade issues.

Precise origins of animals and the contribution of each hunter to the harvest cannot be reliably determined from monitoring at high levels in the trade chain (Box 10). Hunters may hold their catch for later sale or transport their catch long distances from the capture area. Similarly, middlemen transport harvested animals away from their point of origin and allow them to accumulate until it is convenient to sell them.

By monitoring at higher levels of a trade chain, data can be taken quickly from large numbers of specimens, but collection dates and geographic origin will likely be impossible to verify. Nevertheless, the large amounts of data obtained will be useful for analyses of broad harvest patterns and system-wide policies. In addition, in many instances reptile trades may be regulated not at the level of individual hunters but instead by trade controls. Thus, datasets compiled at large processing facilities are useful for measuring the effectiveness of policies aimed at regulating trade patterns. In Argentina and Paraguay, for example, commercial trade in small tegu lizard skins is discouraged at the industry level of trade, not at the level of hunters. Hence, data from tanneries is needed to check compliance with the ban on small skins (Fitzgerald et al. 1994b; Mieres and Fitzgerald 2006).

Clearly, long-term data are needed to draw quantitative inferences about population trends of exploited species. Although we can gain valuable natural and life history information from short-term exploitation data, long-term, time-series data are needed to draw inferences about population trends and effects of harvests on population structure. Depending on the system, biologists trying to interpret patterns from exploitation data may be faced with inherently large amounts of variation in time series. Returning to the example of rattlesnakes, Fitzgerald and Painter (2000) analyzed long-term harvest data, including the average catch per hunter, for Western Diamond-backed Rattlesnakes brought to *rattlesnake roundups*. They found that the number of rattlesnakes harvested, the number of hunters, and the number of rattlesnakes captured per hunter were all extremely variable through time (Figs. 95 and 97A). Despite 39 years of continuous data on rattlesnake harvests, confidence limits around mean rates of long-term harvest were ± 20 percent (Fig. 97B; Fitzgerald and Painter 2000)! They also found that biologically meaningful differences in rattlesnake size were probably due to natural geographic variation rather than to the effects of hunting. In all cases, standard deviations were greater than the means (i.e., mean number of hunters and mean catch per hunter). Because the numerical distribution of hunter contributions to the total harvest is inevitably broad and skewed, measures of hunter effort will also tend to exhibit large variation in relation to the mean. The finding that hunter effort in the rattlesnake system varied widely over time and by locality suggests that the assumption of stable hunter effort when interpreting exploitation data for reptiles is not always met. A modeling study designed to simulate the effect of management interventions on population growth rates in exploited reptiles indicated that relatively low levels of natural stochastic population variation can obscure long-term population trends and make it very difficult to detect whether management practices are affecting the population (Fitzgerald 1994a).

Application of fisheries-type models (Walters 1986; Getz and Haight 1989) to exploitation data for reptiles should greatly enhance our understanding of reptile-exploitation systems, particularly if information on hunter effort can be gathered. To date, harvest models have been applied only to reptile data from several species of crocodilians (e.g., Bradshaw et al. 2006), and sophisticated analyses were possible because of the existence of multiple long-term datasets on crocodilian populations. It will be some time before management programs for exploited herps produce time-series data sufficient for meaningful analyses. Development of sustained yield harvest models for exploited reptiles will be an important area for future research in herpetological conservation.

BOX 10 EXPLOITATION DATA OBTAINABLE AT VARIOUS LEVELS OF A TRADE CHAIN FOR A TYPICAL REPTILE SPECIES, WITH SOME ADVANTAGES AND LIMITATIONS OF MONITORING AT EACH LEVEL

Hunter and Collector

Advantages

- Geographic origin and date of collection can be traced.
- Diet, reproductive state, other natural history information available from skins, carcasses.
- Information on hunting patterns available from working directly with hunters.
- Measures of hunter effort can be devised and implemented.
- Potential to work with hunter networks and local communities.

Limitations

- Small sample sizes.
- Relatively expensive.

Middleman (establishment of check stations)

Advantages

- Trade practices of middleman levels can be studied.
- Efficient data collection at check stations.
- Larger sample sizes.

Limitations

- Precise origin and collecting date of material unknown.
- More difficult to measure hunter effort.
- More difficult to measure hunting patterns.

Final Accumulation Point (e.g., tannery, slaughterhouse, pet warehouse)

Advantages

- Large samples can be measured quickly.
- Body-size distributions and sex ratios are representative of the entire harvest.
- Program-level compliance with size and sex restrictions can be evaluated.
- Information on size of total harvest can be obtained, if monitoring is comprehensive.

Limitations

- Precise origin and collecting date of material unknown.
- No information on hunter effort or information on hunting patterns.
- Inability to work directly with hunters and local communities.

International Wildlife Trade Databases

Advantages

- World-wide trade can be understood.
- Import and export patterns can be studied.

Limitations

- No information on domestic trade.
- Imprecise or ill-defined data in the database are difficult to verify or correct.

Once exploitation data accrue, biologists and statisticians will be able perform some type of time-series analysis to the dataset. Several books devoted to time-series analysis are available, and statistics for time series are included in advanced statistical software packages (e.g., Diggle 1990; Hamilton 1994; Cryer and Chan 2008). Simple linear regressions computed from exploitation data probably do reveal meaningful trends, but time-series data do not meet all the assumptions of linear regression statistics. For example, successive data points in a time series are autocorrelated and not independent (Rasmussen et al. 1993). Fitzgerald (1994a) and Fitzgerald and Painter (2000) log-transformed raw harvest data and calculated long-term harvest rates using the average of log-transformed ratios of values for each variable between years for the numbers of animals harvested, numbers of hunters, and numbers of animals collected per hunter. This method of estimating long-run rates is analogous to estimating long-run population growth rates in a stochastic environment (Tuljapurkar and Orzack 1980; Dennis et al. 1991; Fitzgerald 1994a). A rate of zero means that over the long-term, harvest rate is neither in-

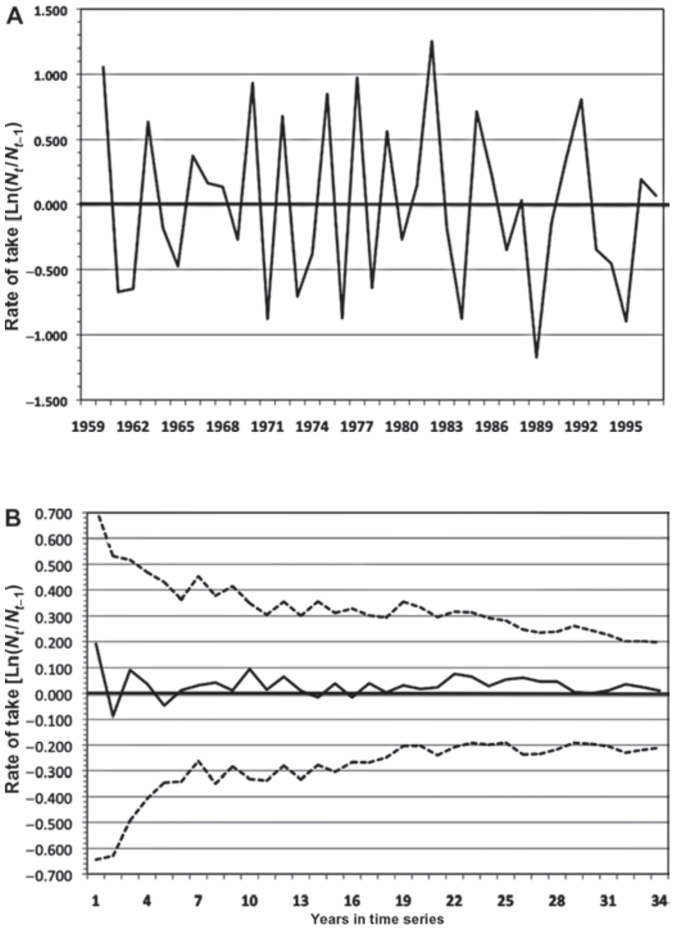

FIGURE 97 Take of Western Diamond-backed Rattlesnakes (*Crotalus atrox*) at the Sweetwater, Texas, Rattlesnake Roundup. (A) Annual rates of take were extremely variable. (B) Annual fluctuations in numbers of rattlesnakes brought to the roundup resulted in long and imprecise convergence to a mean rate of take, despite long-term data. Confidence limits remained very broad, even with all years included in calculation of the mean rate (=0.007 ± 0.204). (From Fitzgerald and Painter 2000; © The Wildlife Society, reprinted with permission.)

creasing nor decreasing. The reliability of rate estimates can be expressed as 95 percent confidence intervals around the long-run mean. Of course, the harvest rate is an average and can be interpreted only over the number of years in the estimate; it is not a forecasting tool.

Informed Conservation of Exploited Species When Data Are Lacking

How should biologists like Haldre and the rest of us grapple with these quantitative challenges in our attempts to interpret exploitation data? This is an especially daunting question given the embryonic stage of management programs for exploited reptiles, lack of long-term data, and the inherent natural variability in population growth rates. Regardless of how few data are available, biologists and managers are called upon to and can make informed decisions about conservation of exploited populations. Best estimates of the magnitude of the trade, together with perspectives on its potential to expand into new areas or to affect similar species that are not yet exploited, can guide conservation priorities. Information on the life histories and geographic distributions of species, the conditions of habitats, and the general patterns of trade and exploitation can be integrated to inform scientifically defensible conservation strategies.

In the hypothetical situation of the Blue-tongued Mango Skinks, Haldre is dealing with a species that is a habitat specialist with a restricted geographic range. There are indications that the market demand is high and that collectors are accessing most of the known habitat. She knows that onset of reproduction in the species is delayed and that egg clutches are small. Thus, despite the absence of long-term trade data and precise demographics, from the perspective of a conservation biologist, recommending strict management of these lizards is a no-brainer.

A real-world comparison of situations surrounding the exploitation of rattlesnake species with different life histories and geographic distributions drives home the message that multiple sources of information can inform the conservation and management of exploited species. Although all three have been subject to hunting and trade, the long-term prospects for conservation of Western Diamond-backed Rattlesnakes, Eastern Diamond-backed Rattlesnakes (*Crotalus adamanteus*), and Timber Rattlesnakes (*C. horridus*) are very different and can be understood without sophisticated harvest models. The Western Diamond-backed Rattlesnake matures relatively early, has a large geographic distribution, and uses many habitats. Hunting for the trade in skins and meat and rattlesnake roundups occurs in an area vastly smaller than the range of the species. Despite decades of unregulated harvest, Western Diamond-backed Rattlesnake populations persist. Thus, solely from the perspective of sustainability, populations of this species are probably not threatened with overexploitation on regional scales. In contrast, the Eastern Diamond-backed Rattlesnake has a more restricted distribution and is hunted over a relatively larger portion of its range. In addition, its habitat is increasingly fragmented, resulting in populations that are more susceptible to hunting and other sources of mortality. Hunting for rattlesnake roundups can exacerbate population declines in already diminished and fragmented populations (Means 2009). In the Timber Rattlesnake, especially in the northern part of its range, the onset of reproduction is delayed, litters are small, females reproduce infrequently, and populations are limited by a scarcity of den sites. These life history traits, in combination with habitat loss, make Timber Rattlesnakes very susceptible to overexploitation (Brown 1993; Brown et al. 1994; Greene 1997). The rattlesnake examples illustrate that we can draw conclusions about prospects for sustainability of reptile populations and make honest and defensible recommendations about the management of reptile species based on a knowledge of their life history traits, the conditions of the habitats they occupy, and their geographic distributions. In addition to population sustainability, however, we must consider the multiple ethical issues associated with the trade in reptiles when formulating modern management plans. Hunting methods, transport and treatment of live animals, public education, and unregulated commercial exploitation are legitimate and serious conservation issues that need reform, especially in the case of exploited rattlesnakes and other wildlife that is traded alive (Weir 1992; Fitzgerald and Painter 2000; Mushinsky and Savitzky 2006; Adams and Thomas 2008; Means 2009).

Designing Monitoring Programs for Exploited Reptiles

The ultimate goal of a monitoring program for exploited reptile species is to assemble long-term time-series data on hunter effort, the size of the total harvest, and on the size distribution and sex ratio of harvested animals. Information should also accrue on human social and economic factors such as markets, trade practices, hunting practices, and ethical treatment of live animals. Challenges to setting up a monitoring program that will achieve these goals include gaining honest access to the reptile trade, developing a network of sites where exploitation data can be gathered, and institutionalizing the program so that monitoring will continue over the long term.

In setting up a monitoring program for exploited reptiles, a means must be devised for confirming the total number of animals harvested. For most exploited species of amphibians and reptiles, systems that allow biologists to measure the total harvest have not been implemented. Resource management agencies of some states in the United States and the U.S. Fish and Wildlife Service (USFWS) compile data on the number of animals in commerce at the national level. Wildlife imports and exports are reported to the USFWS and compiled into the Law Enforcement Management Information System (LEMIS) database. Internationally, CITES permit data compiled by the World Conservation Monitoring Centre (WCMC) provide an idea of the level of global trade for species listed on CITES appendices. Trade databases report numbers of animals in commerce, which can differ from the actual number harvested. Particularly in international markets, the actual harvest may be greater than trade numbers indicate because local trades for the species and excess animals that are harvested and not sold go unreported, as do damaged skins (or other products) that are not exported (Mieres and Fitzgerald 2006). Conversely, the actual harvest may be less than the number traded if traders unload stockpiled skins from previous harvests or the trade contains captive-bred animals. Until regulations and management programs catch up with the need to monitor exploited species, it will be difficult to know the true numbers of reptiles that are harvested. Nonetheless, import/export databases, although potentially incomplete, provide crucial information needed to assess population trends in commercially harvested species.

A good monitoring system should provide demographic data from the harvest at low as well as high levels in the trade chain. Monitoring at the level of hunters or the first level of middlemen provides useful information about hunting patterns, hunter effort, and demographic characteristics of local harvests. Low levels of the trade chain can be monitored by working with a network of hunters or by establishing check stations with cooperators who already are collecting harvested animals from hunters. Data from high levels in the trade (e.g., at tanneries or pet warehouses) provide information about the makeup of the total harvest. It goes without saying that regardless of the level of monitoring, monitors and traders must develop mutual trust and respect.

The tegu lizard monitoring system that operated in Paraguay from 1991 to 1998 included monthly visits to eight check stations at rural trading posts along a major highway bisecting the important hunting areas, coupled with monthly visits to the tanneries where skins were processed and exported. Total harvest numbers were estimated from the legal quota and CITES export permits (Fitzgerald et al. 1994; Mieres 2002). A network of collaborating hunters in the field provided specimens for natural history studies. Monitoring data from the different trade levels were compared to provide insight into trade practices of middlemen, to track geographic variation in demographic parameters of the lizards, and to evaluate the effectiveness of conservation policies at different levels of the trade (Fitzgerald et al. 1994; Mieres and Fitzgerald 2006). Differences in size distributions of skins from check stations and at tanneries were apparently caused by middlemen stretching the skins they purchased before reselling them. Compliance with the ban on commercialization of small skins was good.

Commercial harvests can also be monitored at midlevel collection points. For example, in several states rattlesnake harvests are monitored at traditional rattlesnake roundups, which essentially function as check stations. A similar system was established for tegu lizards in Argentina. Wildlife authorities from each province gathered data on the lizards at warehouses where traded skins accumulated before being sold to tanneries.

When designing a monitoring system, biologists should carefully consider the availability of personnel, vehicles needed to transport the monitoring team, and realistic levels of funding to support the monitoring program over the long term. Although it would be ideal to monitor the harvest at several levels of the trade chain with frequent repeat visits to many sites in the field, it is more important to design a monitoring system that has a good chance of persisting long into the future. In most instances, long-term data from a few sites will prove more valuable than a few seasons of data from many sites. Therefore, check stations, where data on the harvested animal will be collected, should be located at sites that are accessible throughout the monitoring season and likely to produce data year after year.

Monitoring programs for exploited species should employ simple monitoring protocols that enable large numbers of harvested animals to be processed quickly. A team of monitors working together at a check station measures harvested animals and records data on standardized sheets. Locality and date should be noted each time monitoring information is gathered. Size (snout-vent length, SVL) of the animals or skins should be measured, and sex determined if possible. For species that are exploited for their skins, such as tegu and monitor lizards, the length and width of dried skins should be measured. Width is an important variable because commercial trades in reptile skins are often based on skin width. The relationship between the length and width of a dry skin and a particular SVL can be quantified with linear regression analyses and used to estimate SVLs of the animals from which skins were taken (Fitzgerald 1990; Shine et al. 1996; Mieres and Fitzgerald 2006). Size frequency distributions and sex ratios of harvested animals can be constructed for each monitoring site visited. Datasets replicated over standardized time intervals provide time series that can be analyzed statistically and used in various harvest models.

It is important to distinguish data collected as part of natural history studies of species from those obtained when monitoring exploited populations. Natural history information is needed to understand the ecological and life history traits of a species that may underlie its prospects of sustainable utilization (Fitzgerald et al. 1993, Shine, Ambariyanto et al. 1999a). Once known, however, the life history traits and morphometrics of the animals in a population do not need to be monitored continuously. Rather, studies of diet, reproduction, morphological variation, and geographic variation, for example, are best undertaken as research projects, distinct from the monitoring program. Biologists may be tempted to measure an extensive suite of morphological variables from exploited reptiles whose basic biology is poorly known, but superfluous data collection can be distracting and time consuming. Measuring body size and noting sex takes a few seconds, whereas measuring various morphological traits and reproductive variables and recording stomach contents add significant work and time. Additionally, once morphological variation has been described based on statistically robust samples, not much is gained by monitoring a species' morphology. It is a good idea to make vouchers from whole specimens obtained from the harvest, with tissues preserved for genetic analysis. Once these specimens have been catalogued in a natural history collection, they will be accessible to biologists well into the future and used for many kinds of natural history and systematic studies.

Acknowledgments

I thank Mercedes Foster for her encouragement to create this chapter. I also thank Gordon Rodda for letting me build upon his informative hypothetical scenario of the ficticious Blue-tongued Mango Skink in Chapter 15.

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PART FOUR

CONCLUSIONS

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CHAPTER SEVENTEEN

Reptile Biodiversity: Where Do We Go from Here?

ROY W. McDIARMID and MERCEDES S. FOSTER

> I am impressed by the fact that we know less about many
> modern [reptile] types than we do of many fossil groups.
>
> ALFRED S. ROMER, 1956

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Introduction

As the human population increases, more demands are made of the environment for natural resources and for space in which to live and grow food. As a result, in many areas forests are overharvested or clear cut, ecosystems polluted, resources of all sorts depleted (a lack of water is likely to precipitate the next global environmental crisis), and the earth warms at an alarming rate. As natural environments are affected, so too will be the organisms that occupy them; some species will decline or go extinct, populations of others will increase, and some will adapt to new conditions or move to more amenable places. Among the organisms that are affected will be species that provide a variety of direct benefits to humans (i.e., species that are eaten, serve as sources of building materials, fiber, medicines, etc.). Such organisms will likely be the first recipients of conservation or management efforts to mitigate the effects of habitat alteration and maintain populations. Populations of some other species will survive in ecosystems maintained because of the indirect services they provide in cleaning the air, replenishing aquifers, and enriching the soil. Most organisms, however, will not fit into these categories. What will happen to them? Where do reptiles fit in this scenario?

To stave off the negative effects of potential population changes, increases and declines, we must act to ensure that organisms on which we depend will not disappear nor will new ones that do us harm overwhelm us. In the best of worlds, we hope to be aware of potential serious changes before they reach a tipping point, and populations cannot be resuscitated. We do this by inventorying species so that we know what occurs where, and then we monitor their populations to determine the magnitude of change and whether it is part of a normal population cycle or a serious perturbation.

Our intent in producing this handbook is to provide investigators with a single source through which they can access a set of recommended standard methods and appropriate analytical tools to inventory and monitor reptile diversity anywhere in the world. The use of standard methods increases the comparability of data from site to site and through time, thereby reducing the time, effort, and other resources required to obtain a reasonable idea of population sizes of reptile species throughout the world. Once that information has been obtained and the baseline data are made available, it should be possible to recognize population changes early on and deal with them before species numbers decrease to critical levels. Certainly, that is the point at which we want to be. But once we get there, one asks, "Now what?" "What measures can we take?" "What should we do?"

Although the world has long been aware of the need to protect and manage wildlife, very little attention has been paid to reptiles. Beginning with the initial workshop in 2002 to organize this volume and continuing through the early stages of assembling the contributions for this book, we were struck by the general consensus among participants that, with a few exceptions, reptiles had generally been ignored by the world conservation community over the past 40 years. While lizards and snakes have rarely caught anyone's attention, multiple important conservation and management initiatives have been directed at turtles, especially the marine forms, and crocodilians, which together constitute less than 4 percent of reptile diversity. What is it that made them so attractive to conservation efforts? Why have those conservation efforts been so successful? What can we learn from them that might be directed toward lizards and snakes? Here we briefly review some of these initiatives and point out some current activities relative to the inventory and monitoring of reptiles throughout the world.

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Major Conservation Efforts

Sea Turtles

With the establishment in 1969 of the Marine Turtle Specialist Group under the auspices of the IUCN (now World Conservation Union) in Switzerland, the sea turtle research and conservation community began moving in a direction new for the herpetological community by emphasizing research on the status of one group of reptile species and populations. By 1975, all seven species of sea turtles had been listed under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which restricted their commercial trade. Soon after (1978), six species received protection under the U.S. Endangered Species Act. Several other activities initiated at about the same time also played a role in mobilizing the sea turtle community. First, the *Marine Turtle Newsletter* was started (August 1976) as a mechanism to keep the community informed about advances in basic and applied sea turtle research, new techniques for marking these turtles and tracking their movements, and legal decisions affecting their statuses. Today, it is available quarterly as an online publication (http://www.seaturtle.org/mtn).

Second, the sea turtle community began holding an annual meeting devoted to sea turtles and published the proceedings in the *Technical Memorandum Series* of the National Marine Fisheries Service (NMFS), National Oceanographic and Atmospheric Administration (NOAA). The research findings made available in these volumes have done a great deal to broaden our understanding of sea turtle biology and to advance the conservation of these animals.

Other Turtles

A program similar to that on sea turtles and also associated with the IUCN is the Tortoise and Fresh Water Turtle Specialist Group (TFTSG; http://www.iucn-tftsg.org/). The Group has about 250 members who work actively to identify and document threats to all species of tortoises and freshwater turtles and to help catalyze conservation action to ensure that none becomes extinct and that sustainable populations of all species persist in the wild. The TFTSG is closely associated with the Chelonian Research Foundation (CRF), a nonprofit organization that publishes a peer-reviewed professional scientific journal, *Chelonian Conservation and Biology*; a monograph series, *Chelonian Research Monographs*; and the *Turtle and Tortoise Newsletter.*

Crocodilians

The community of crocodilian biologists followed a similar path and formed the Crocodile Specialist Group (CSG; http://www.iucncsg.org/ph1/modules/Home/) in 1971. The CSG comprises a worldwide network of biologists, wildlife managers, government officials, independent researchers, farmers, traders, tanners, leaders of the fashion industry, and representatives of NGOs and private companies. They are actively involved in the conservation of all species of crocodilians in the wild. Members of the CSG network advise governments and wildlife management agencies, evaluate the conservation needs of crocodilian populations, survey wild populations, initiate research projects and conservation programs, and provide technical information and training. CSG members present their work and exchange ideas at biennial working meetings; the proceedings of the meetings are published.

When the CSG began, all 23 species of crocodilian were endangered or threatened; hunting for hides had reduced wild populations of some species (e.g., *Crocodylus porosus*) by more than 95 percent. Following extensive population surveys in various regions (e.g., Messel, Vorlicek, Elliot et al. 1981) and other research efforts, treatment of crocodiles began to change. Some populations were protected, and the unregulated harvest of hides slowed or stopped. The possibility of sustainable use emerged, and by 1996, one-third (8) of the species were sufficiently abundant to support well-regulated annual harvests, one-third (8 species) were no longer in danger of extinction but were not yet sufficiently abundant to harvest, and one-third (7) remained endangered. By the beginning of the 21st century, many programs involving consumptive use were in place; the earnings generated by economic use, in turn, resulted in increased investment in conservation (see Webb 2000). This remarkable improvement in the status of a group of very large reptiles (e.g., saltwater crocodiles in Northern Territory of Australia; Fukuda et al. 2011) that had been heavily hunted for their hides is a tribute to the efforts of a dedicated group of crocodile biologists and the CSG. The latter group argues that the key to this success was the cooperation of companies involved in the international reptile skin and leather trade, crocodilian farmers and ranchers, skin traders, tanners, manufacturers, fashion designers, and major retailers. The CSG continues its efforts to improve and protect populations of the seven endangered crocodilian species and certain threatened populations of more abundant species.

Other Reptiles

The relatively new Iguana Specialist Group (ISG; http://www.iucn-isg.org/index.php) focuses primarily on large iguanas in the West Indies. Its purpose is to help design and implement effective conservation measures for these lizards. The group also publishes a newsletter, which first appeared in the fall of 1998. We know of no other taxonomically organized reptile group that has identified inventory, monitoring, and conservation activities as its primary interests.

In the late 1970s, individuals concerned about range-wide declines of two species of burrowing tortoises of regional importance in the United States formed active conservation groups focused on these species, the Desert Tortoise (*Gopherus agassizii*) and the Gopher Tortoise (*Gopherus polyphemus*). The Desert Tortoise Council (http://www.deserttortoise.org/), established in 1976, promotes the conservation of the Desert Tortoise in the southwestern United States and adjacent Mexico; it publishes the proceedings of its annual meetings. The Gopher Tortoise Council (GTC; http://www.gophertortoisecouncil.org/), formed in 1978, offers professional advice on the management, conservation, and protection of Gopher Tortoises; encourages and funds research on their life history, ecology, and management; offers public education programs; and promotes protection of this and other upland species in the southeastern United States. Each group has effectively promoted tortoise conservation initiatives in its region of the country.

Some important edited volumes on lizards that were derived from symposia held at meetings of professional herpetological societies in the United States were published in the 1980s (e.g., Burghardt and Rand 1982, on Iguanas; Estes and Pregill 1988, on lizard relationships). Campbell and Brodie (1992) published the results of a symposium on pitviper biology. That symposium and the subsequent publication of its proceedings energized other snake biologists to publish another volume on the biology of vipers (Schuett et al. 2002); that book was followed by third volume dealing only with rattlesnakes (Hayes et al. 2008). These viper volumes devote considerable space to natural history and, notably, include sections on conservation ecology and education. A volume by Henderson and Powell (2007) on boa and python biology is another important contribution to our knowledge of "giant" snakes.

All of these volumes have made valuable contributions to our understanding of the distributions and natural histories of important subsets of reptiles. On the other hand, we know little or nothing about thousands of other species scattered across the globe. Why have they not received similar attention? A review of the species that have been the focus of significant attention is revealing.

Species Attributes

Targeted Groups

Interestingly, the groups that have received the most attention seem to share three attributes. First, they have an economic connection—either a positive one that people wish to continue to exploit (e.g., the harvest of crocodilians for their skins or turtles and their eggs for food or presumed medicinal purposes) or a negative one that they want to avoid (e.g., sea turtles tangled in fishing nets, which can lead to fines or fishing prohibitions in some areas). Consequently, people far beyond the average conservationist, nature lover, or scientist have expressed interest in sea turtles and crocodilians, which has significantly increased support for research and management, be it political, financial, or sweat equity. Second, they are relatively easy to locate, census, and study, if not year round, then at least at certain times or in certain situations (e.g., sea turtles on nesting beaches, tortoises in burrows, crocodile nests or individuals basking on river banks). And third, their species diversity is low enough to be tractable.

Consider sea turtles. There are only seven species, and they are all large and easily recognized. Females return to sandy natal beaches at predictable times to dig nests and lay their eggs. Investigators do not need to spend time searching for their study animals, which can be located, captured, marked, and counted with little difficulty. Population size, female fecundity, hatching success, recruitment, and many other life history traits can be estimated at the same place in a single field season. Accordingly, data are amassed at a good pace, allowing for better informed decisions about managing sea turtle populations, with higher expectations of success. Such opportunities are rare or nonexistent for most other species of reptiles.

The situation with the crocodilians is similar. The number of species is low, and the animals are large and usually easy to observe and count. In contrast to sea turtles, however, crocodilians are dangerous and can eat people, pets, and livestock. Not surprisingly, they have frequently been targeted for removal. However, the hides of many species are a valuable commodity, which makes them attractive to people with other interests, including hide hunters, skin processors, and members of the leather industry. The demand for skins has also provided an incentive for ranching. Whereas an increase in crocodilians in an area was viewed negatively by many people in the past, it is now perceived as a measure of success, and considerable energy and resources are directed toward protecting these animals. Leather producers, leaders in the fashion industry, and others with commercial interests in crocodilians are actively involved in their conservation, often with considerable success. In some ways, venomous snakes are similar to crocodiles because of their value for medical research; venoms, in particular, may have significant potential for treating a number of debilitating human conditions. In addition, because of their dangerous nature, they are often better known and better studied than most of the smaller, nonvenomous species of snakes. Although the characteristics shared by species that have been the focus of conservation programs have certainly contributed to the success of those programs, one additional factor has been critical. That is the high interactivity among members of the groups that carry out these programs. They hold workshops and meetings to exchange information, to discuss and consult about problems that have arisen, and to identify needs and topics of potentially fruitful research. In addition, they communicate with their members (partners in the endeavor) through published proceedings and newsletters. The groups are visible, organized, and have the support of the IUCN and the Species Survival Commission, which itself communicates with the interested parties through research publications and newsletters. All of these activities help to create and maintain an informed, interested constituency without which none of these projects could succeed.

Neglected Groups

A review of reptile groups that have not been the focus of conservation/management programs also points to some common patterns. First, many of the 8,736 species of Sauria are much smaller than turtles and crocodilians and generally more difficult to locate, especially those species that burrow, live in the rainforest canopy, or are entirely nocturnal. Once individuals have been captured, however, they can be fitted with radio transmitters that will allow an investigator to relocate and follow them. Improvements in the designs of both transmitters and power sources have led to significant increases in transmission distance and radio life, and not surprisingly, transmitters have already been used in studies of crocodilians, turtles, and snakes (e.g., Greene 1997). Advances in the miniaturization of telemetry transmitters have been even more remarkable. A model weighing 0.2 g (including the power cell), with a life of about 3 weeks, should be appropriate for an 8- to 10-g reptile. Information on the specifications of different types of transmitters is available on the websites of most manufacturers and retailers of telemetry equipment (also, see Naef-Daenzer et al. 2005). The numbers of species of snakes and lizards, which are overwhelming, also present a problem. Keeping track of 24 species of crocodilians is surely more manageable than trying to monitor 3,217 species of snakes, or even 652 species of colubrid snakes. In fact, it would be downright easy (at least in a comparative sense), as demonstrated by reports from the CSG.

Finally, very few snakes or lizards generate the obvious economic or human interest that crocodilians and sea turtles do. Is there a solution? Are there ways to generate as much interest in these "other reptiles" as there is in the groups that are already the subject of attention? From an economic standpoint, there may be a few possibilities. When it became difficult for designers and the fashion industry to obtain crocodile and alligator skins, many leather producers sought substitutes, including snake (python) and lizard (two species of tegu, *Tupinambis* spp.) skins that could be used to meet the demand for exotic leather accessories (e.g., cowboy boots). Because the products are rather esoteric and tend to be expensive, the demand for these goods can be fickle, depending on the current fashion trend. Consequently, the harvest of the alternative skins appears to have been driven by market demand, rather than by the ability of hunters to harvest the skins; nevertheless, local people did and do reap an economic benefit, although it may be intermittent and irregular.

Another possible economic generator is the pet trade. Demand for "exotic" reptiles as pets is enormous, especially in the United States, throughout Europe, and in Japan (Christy 2010). For example, the United States is reported to have imported just under 2 million live reptiles in 2001 (World Wildlife Fund 2010), and numbers have increased since then. The value of those imports is equally large. Franke and Telecky (2001) estimated the value of the retail trade in live reptiles, amphibians, and related products in the United States to be at least 2 billion dollars annually, and it is likely that the amount has increased since then. If the trade in wild-caught animals were to dry up (restricted by governments, endangered species treaties, or other similar controls) or the animals became rare enough, their prices could soar, and people might want to ensure their continued availability, perhaps through captive breeding. If this were to happen, it might provide an issue that would galvanize aficionados of reptile pets and give rise to a political and economic force in support of a reptile conservation movement, much as members of the Audubon Society are for birds.

On the other hand, there is also an active movement in the United States to discourage the pet trade in reptiles, which are considered by some to be fragile animals not at all suited to captivity, even though many are captive bred. To their credit, more and more individuals are preparing books, manuals, and websites to train pet keepers to care for their animals properly and to treat them humanely (from a reptile's point of view). We hope that these groups will make the effort to bridge the gap between them and look for solutions that will satisfy both their interests. Programs along these lines have also been developed with amphibians (e.g., Tree Walkers International, http://www.treewalkers.org/).

What about those species that really are numerous and that, one hopes, will continue to be so? Again, we can take a hint from the turtle, crocodilian, and iguana specialists' groups and identify smaller, more tractable subsets within the diversity of snakes and lizards—something that can and should be done by the people who care the most about particular reptiles. In fact, such self-organizing is already going on, and the history of the trade in the Ball Python (*Python regius*) provides an interesting example of it (e.g., see Murphy and McCloud 2010 and Gorzula et al. 1997 for detailed coverage). As certain species, varieties, or morphs of reptiles are identified as desirable for the pet trade, skin trade, or as food for local people, an enterprising group might provide funding to determine the status of their populations, investigate their life history traits and habitat needs, and attempt to set up a sustainable use program for some populations. Harvested animals could be used for food, skins, or the pet trade. The program would involve local people in a way that would provide them with a direct benefit and a stake in ensuring protection of the species' habitats and conservation of their populations. Perhaps mainland populations of Green (*Iguana iguana*) and Spiny-tailed (*Ctenosaura* spp.) iguanas might be worth investigating in this respect. The Iguana Specialist Group would be in a position to coordinate and advance such activities.

Where We Are Today

Lately, interest in biodiversity has been increasing, and it seems to be embracing all groups. The trade in exotic reptiles has increased dramatically, and while we applaud the increased interest in the group, it is also driving some of our concerns about the threats to many of these desirable, valuable species that this interest may entail. Unfortunately, sound population survey data and demographic information are often lacking, making it difficult to identify declining species early on. Even after a decline has been identified, however, it is difficult to act; natural history data are paramount as a basis for developing conservation or management plans so that extinction can be averted. Yet more often than not, those data have yet to be collected. It is ironic, as some of our colleagues have noted, that getting a license to export species for the pet trade is often far easier than getting a collecting permit to learn about their ecologies and life histories.

Despite the somewhat gloomy outlook for biodiversity conservation, positive things are starting to happen. One encouraging development is the significant increase over the past several decades in the cadre of trained herpetologists throughout the world (and especially in what was previously referred to as the "Third World"). The training of students and development of local expertise continue at increasing rates in many of these countries, to the benefit of all. Twenty-five or more years ago, most studies of the systematics and ecology of reptiles in tropical areas were done (with a few notable exceptions) largely by investigators from countries in temperate regions. Today, much of that research is conducted by local scientists, and in most cases, they are leading the way for all.

We also note that the number of outlets for and publications of descriptions of new species (e.g., Zootaxa [http://www.mapress.com/zootaxa/]) and faunal lists (e.g., Checklist [http://www.checklist.org.br/]) has increased dramatically in the last 10 years. In South America, two new journals devoted to herpetology have appeared in the last decade. This trend is apparent in the rest of the world as well. The increase in the number of regional herpetofaunal studies published over the past 20 years, especially for Asian countries, is remarkable (see source references in Chapter 2, "Reptile Diversity and Natural History: An Overview"), and their rate of appearance continues unabated. Such studies reflect an increase in sampling in poorly known areas, a general expansion of our understanding about the distributions of species, and an increase in scientific specimens for study in museum collections. The increases in knowledge and in the availability of specimens facilitate more detailed regional studies of reptile ecology and natural history (e.g., see Seburn and Bishop 2007) and can lead to more directed studies on the interactions between reptiles and people (e.g., Mitchell et al. 2008). Certainly the completion this year of the 22-volume work titled *Biology of the Reptilia* by Carl Gans and 15 co-editors (1969–2010)[1] provides a reference on reptiles that is unrivaled in publishing history and should make our effort to elucidate reptile distributions and diversity easier. At the same time, volunteer organizations such as Partners in Amphibian and Reptile Conservation (PARC [http://www.parcplace.org/]) sponsor programs and mobilize the volunteers necessary to document species occurrences and estimate densities. In 2010, the U.S. Army Corps of Engineers published a set of guidelines for inventorying amphibians and reptiles on Corps lands (Guilfoyle 2010). Recently, we learned about exciting new training and research projects being initiated in the Peruvian Amazon by the International Expeditions Conservancy (www.Ietravel.com). All of these developments give us hope.

Even with these positive trends, several aspects of our science and the study of reptile diversity are disturbing. Fewer and fewer students are electing to study systematics, and the number of jobs for systematists and herpetologists has continued to decline. Increasing numbers of universities emphasize laboratory or mathematical-modeling approaches to science; fewer and fewer "-ology" courses (herpetology, mammalogy, ornithology, entomology, etc.) and fewer hands-on field courses that explore ecology and natural history are being taught. We hope this volume will reinvigorate the academic and conservation communities and persuade them to be more supportive of organism-focused courses, including those on reptiles, and to provide students with more opportunities for field experience so that they learn can about this important component of our natural diversity.

In fact, learning about biodiversity and the natural world should begin long before university. Students who are not exposed to these fascinating creatures in their natural environments suffer a misfortune exacerbated by the increasing urbanization of human populations, loss of natural habitats, and development of cultures in which a Saturday trip to the mall displaces a hike in the hills. People are still afraid of snakes (and lizards), but they can learn—if we will teach. We all need to get involved. So, give a talk at your child's school (and take along a live snake that the students can hold), take a scout troop on a hike, talk to a local PTA (parent-teacher association) or civic organization once or twice a year, or give an interview for the local newspaper or on the radio about the importance of this work (the rest of the time, do inventories.)

Data on the global diversity of reptiles and their distributions will be a gold mine of information from an intellectual viewpoint. They will provide the raw material for many studies and analyses aimed at answering a multitude of stimulating questions, including why and how reptiles reached the high levels of diversity that we see. All such work is important, and certainly the insights gained will be useful for any project on reptiles. However, our primary goal in this time of increasing human populations and decreasing habitat and biodiversity must be to use the information in a more practical way, that is, to ensure the persistence—worldwide—of viable populations of as many species of reptiles as possible.

The Future

This volume contains a set of recommended standard methods and analytical tools that are appropriate for studying reptile diversity anywhere in the world. It provides the information necessary to carry out a large-scale inventory project or a local monitoring program, as well an entrée into additional literature for dealing with any special situations encountered. As we argued previously, we believe that standard methods are the best approach to spatial and temporal comparisons among reptile species. We also realize, however, that some of the techniques we advocate can be modified to improve their effectiveness, especially as we learn more about the targeted habitats and species and as investigators strive to answer new questions and pursue new knowledge. When changes to a technique truly improve the results obtained, we encourage the publication of such modifications or improvements and the development of suitable analytical techniques to allow comparisons of new data with those obtained in previous studies. We also ask investigators to send us such information so that it can be incorporated into subsequent editions of this book or to publish it so that it will reach other researchers in a timely fashion.

As national programs develop and data accumulate, national or regional centers could serve as repositories for baseline data obtained with these methods and thereby facilitate spatial comparisons of reptile diversity across habitats, ecosystems, and landscapes and provide temporal insights into population dynamics in regions likely to be affected by climate change. Only long-term monitoring and sampling programs can provide the data needed to prioritize specific areas for long-term protection and to make other types of wise land-use decisions that ensure the continued existence of reptiles as part of our natural heritage.

When we started this project we were motivated by our concern about the lack of baseline data for reptiles and their importance in natural systems. We also recognized the relative success afforded by the previous book on amphibian inventory and monitoring techniques, and the potential for having a significant positive impact on the conservation of reptiles and their habitats. In spite of the innate fear engendered by some forms and the venomous and dangerous characteristics of others, reptiles are among the most important groups of organisms on earth, and they play a significant role in the natural functions of our environment. How effective we are in dealing with their conservation needs, some of which are quite complex, will depend in a large part on our ability to gather baseline data on their distributions and population densities and to predict their responses to the ongoing and increasing anthropogenic intrusions into their life histories and natural environments. We believe that our ability to predict these changes will depend on an increased understanding of the natural histories of all reptiles, and we are hopeful that this book will provide needed impetus to that end.

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[1] The series Biology of the Reptilia, volumes 1–22 (1969–2010). Edited by Carl Gans, with individual volumes co-edited by K. Adler, A. d'A. Bellairs, F. Billett, D. Crews, W. R. Dawson, K. A. Gans, A. S. Gaunt, R. B. Huey, E. A. Liner, P. F. A. Maderson, R. G. Northcutt, T. S. Parsons, F. H. Pough, D. W. Tinkle, and P. S. Ulinski. Academic Press, San Diego, California (1969–1982); John Wiley & Sons, New York (1985); A. R. Liss Inc., New York (1987); University of Chicago Press, Chicago, Illinois (1992); and Society for the Study of Amphibians and Reptiles, Ithaca, New York (1998–2010).

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APPENDIX I

Selected Institutions with Significant Collections of Reptiles

MERCEDES S. FOSTER and ROY W. McDIARMID

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Introduction

The importance of collections of scientific specimens to both research and society has been widely acknowledged (e.g., see IWGSC 2009). Studies based on such collections can have impacts in areas as diverse as education, the economy, the environment, agriculture, public health, and national security. As new technologies develop, collections undoubtedly will have critical new uses in areas as yet unimagined.

One of the most significant sources of items making up scientific collections, both in the past and at present, are specimens collected in conjunction with scientific studies, particularly biodiversity surveys. Biological specimens collected as reference materials for species identifications and documentation of occurrences are called vouchers. The value of these vouchers and the multitude of other uses they have are discussed in detail in Chapter 6. Because of the importance of this material, Reynolds and McDiarmid recommend (see Chapter 6, p.89) that "voucher specimens and their associated data . . . be transferred to a permanent repository as soon as practical after collection in order to minimize opportunities for loss or deterioration." They also provide a number of criteria that institutions should meet if they are to serve as repositories.

The resources required to maintain a museum collection are substantial. Consequently, institutions must often be selective in the collections they can accept. Some museums restrict their holdings to specimens of certain types (e.g., skeletal materials or fluid-preserved), certain taxa (e.g., turtles), or certain geographic areas (e.g., Southeast Asia or Spain). We recommend that researchers select an appropriate repository and discuss terms of a donation with museum personnel prior to beginning field work.

To facilitate the identification of appropriate repositories, we provide in this appendix the names and contact information for a number of institutions. The list is not exhaustive, but we have attempted to provide broad geographic coverage and to include most institutions with large collections of reptiles. Most countries also have smaller, local or regional institutions whose collections are used primarily for education and public outreach.

We have listed the URL for each institutional website, if a site is available. Most of the websites provide information about the institution, the collections (e.g., geographic and taxonomic representation), policies regarding specimen use and investigator visits, as well as names and contact information for curators and collection managers. Some websites provide direct access to specimen records and associated data. In addition, natural history museums provide researchers with opportunities to interact with local scientists, develop collaborations, encounter students, and find individuals who may be interested in working as field assistants.

Natural History Museums and Other Specimen Repositories

ARGENTINA

Museo Argentino de Ciencias Naturales "Bernardo Rivadivia"
Zoología de Vertebrados, División Herpetología
Buenos Aires
http://www.macn.gov.ar/cont_Gral/home.php

Museo de La Plata
División Zoología Vertebrados
La Plata, Buenos Aires
http://www.fcnym.unlp.edu.ar/abamuse.html

Fundación Miguel Lillo
Dirección de Zoología
Instituto de Herpetología
San Miguel de Tucumán
http://lillo.org.ar/content/blogcategory/50/81/

AUSTRALIA

Australian Museum
Sydney, NSW
http://australianmuseum.net.au/

Australian National Wildlife Collection
CSIRO
Crace, Canberra
http://www.csiro.au/places/ANWC-profile.html

Queensland Museum
Biodiversity collections
Brisbane
http://www.qm.qld.gov.au/

South Australian Museum
Adelaide
http://www.samuseum.sa.gov.au/page/default.asp?site=1

Museum Victoria
Melbourne
http://museumvictoria.com.au/

Western Australian Museum
Perth
http://en.wikipedia.org/wiki/Western_Australian_Museum

AUSTRIA

Natural History Museum/Naturhistorisches Museum
Vienna
http://www.nhm-wien.ac.at/

BELGIUM

Königliches Museum für Zentral Afrika
Tervuren
http://www.africamuseum.be/home

BRAZIL

Ministério da Ciência e Technologia
Museu Paraense Emílio Goeldi
Belem
http://www.museu-goeldi.br/
http://www.museu-goeldi.br/Nota%20Técnica%20Coleções%20MPEG%20_Conselho.pdf

Museu Nacional, Rio de Janeiro
Sector de Herpetología
http://www.museunacional.ufrj.br/
http://herpetologia-mn.com/index.html

Universidade de São Paulo
Museu de Zoologia
Sao Paulo
http://www.mz.usp.br/

CANADA

Canadian Museum of Nature
Ottawa
http://nature.ca/en/home

Royal Ontario Museum
Toronto
http://www.rom.on.ca/collections/index.php

CHILE

Museo Nacional de Historia Natural
Colecciones Naturales – Zoológicas
Santiago
http://www.dibam.cl/historia_natural/

COLOMBIA

Universidad Nacional de Colombia
Instituto de Ciencias Naturales
Facultad de Ciencias
Museo de Historia Natural
Bogotá
http://www.ciencias.unal.edu.co/

COSTA RICA

Universidad de Costa Rica
Escuela de Biología
Museo de Zoología
San Pedro, San José
http://museo.biologia.ucr.ac.cr/Principal.htm

DENMARK

University of Copenhagen
Zoological Museum
Natural History Museum of Denmark
http://zoologi.snm.ku.dk/english/

ECUADOR

Museo Ecuatoriano de Ciencias Naturales
División de Herpetología
Calle Rumipamba y Ave. de Los Shyris
Quito

Pontificia Universidad Católica del Ecuador
Facultad de Ciencias Exactas y Naturales
Museo de Zoología, Sección de Vertebrados
http://www.biologia.puce.edu.ec/natura.php?c=674

Escuela Politécnica Nacional
Instituto de Ciencias Biológicas
Centro de Zoología de Vertebrados
Museo de Historia Natural Gustavo Orcés
http://www.epn.edu.ec/index.php?option=com_frontpage&Itemid=1

FRANCE

Muséum national d'Histoire naturelle
Zoologie
Paris
http://www.mnhn.fr/museum/foffice/transverse/transverse/accueil.xsp?cl=en

GERMANY

Museum für Naturkun de Berlin (Berlin Museum of Natural History)
Humboldt-Universität zu Berlin (Humboldt University)
Berlin
http://www.naturkundemuseum-berlin.de

Zoological Museum of Hamburg
University of Hamburg
Hamburg
http://www.uni-hamburg.de/biologie/BioZ/zmh/index_e.html

Senckenberg Research Institutes and Natural History Museums
Senckenberg Gesellschaft für Naturforschung
Dresden, Frankfurt, and Gorlitz
http://www.senckenberg.de/root/index.php?page_id=71&PHPSESSID=voqo13kbk4bu22momi9emp50c1k1ovf5

KENYA

National Museum of Kenya
Directorate of Research and Collections
Zoology Department, herpetology section
Nairobi
http://www.museums.or.ke/component/option,com_frontpage/Itemid,1/

MÉXICO

Universidad Nacional Autónoma de México
Instituto de Biología
Departamento de Zoología
Colección Nacional de Reptiles y Anfibios
http://www.ibiologia.unam.mx/zoologia/html_09/index.html

Universidad Nacional Autónoma de México
Museo de Zoología "Alfonso L. Herrera"
Laboratorio de Herpetología
http://mzfcherpetologiaunam.blogspot.com/

THE NETHERLANDS

Naturalis
National Museum of Natural History of the Netherlands
(Nationaal Natuurhistorisch Museum)
Leiden
http://www.naturalis.nl/

PARAGUAY

Museo Nacional de Historia Natural
Sucursal 19, Central XI
Campus, San Lorenzo

PERÚ

Universidad Nacional Mayor de San Marcos
Museo de Historia Natural
División de Zoología
http://museohn.unmsm.edu.pe/index.php#

RUSSIA

Zoological Institute
Russian Academy of Sciences
Division of Terrestrial Vertebrates
St. Petersburg
http://www.zin.ru/lab_e.htm

SINGAPORE

National University of Singapore
Raffles Museum of Biodiversity Research
Department of Biological Sciences
Faculty of Science
http://rmbr.nus.edu.sg/

SPAIN

Museo Nacional de Historia Natural
Madrid
http://www.mncn.csic.es/home800.php

SWEDEN

Gothenburg Natural History Museum
Göteborg
http://www.vastarvet.se/Kultur_Default.aspx?id=41708

Swedish Museum of Natural History
(Naturhistoriska riksmuseet)
Department of Vertebrate Zoology
Stockholm
http://www.nrm.se/en/frontpage.16_en.html

SWITZERLAND

Muséum d'Histoire Naturelle
Département d'herpétologie et d'ichtyologie
Genève
http://www.ville-ge.ch/mhng/departements.php

Naturhistorisches Museum
Basel
http://www.nmb.bs.ch/home.htm

UNITED KINGDOM

Natural History Museum
Zoology collections
London
http://www.nhm.ac.uk/research-curation/

UNITED STATES

Academy of Natural Sciences
Center for Systematic Biology & Evolution
Philadelphia, Pennsylvania
http://www.ansp.org/

American Museum of Natural History
Department of Herpetology
New York
http://research.amnh.org/vz/herpetology/home

Brigham Young University
Monte I. Bean Life Science Museum
Reptiles and Amphibians Research Collections
http://mlbean.byu.edu/ResearchCollections/Collections/ReptilesandAmphibians.aspx

California Academy of Sciences
Department of Herpetology
San Francisco, California
http://research.calacademy.org/herp

Carnegie Museum of Natural History
Department of Amphibians and Reptiles
Pittsburgh, Pennsylvania
http://www.carnegiemnh.org/herps/index.html

Field Museum
Division of Amphibians and Reptiles
Chicago, Illinois
http://www.fieldmuseum.org/research_collections/zoology/default.htm

Florida Museum of Natural History
Division of Herpetology
University of Florida
Gainesville, Florida
http://www.flmnh.ufl.edu/herpetology/

Louisiana State University
Museum of Natural Science
Baton Rouge, Louisiana
http://appl003.lsu.edu/natsci/lmns.nsf/index

Museum of Comparative Zoology
Harvard University
Cambridge, Massachusetts
http://www.mcz.harvard.edu/Departments/Herpetology/index.html

Natural History Museum of Los Angeles County
Department of Herpetology
Los Angeles, California
http://www.nhm.org/site/research-collections/ichthyology-herpetology

U.S. National Museum of Natural History
Division of Amphibians and Reptiles
Washington, D. C.
http://vertebrates.si.edu/herps/

University of California
Museum of Vertebrate Zoology
Berkeley, California
http://mvz.berkeley.edu/General_Information.html

University of Kansas
Natural History Museum
Lawrence
http://naturalhistory.ku.edu/

University of Michigan
Museum of Zoology
Ann Arbor, Michigan
http://www.ummz.lsa.umich.edu/rep_amph/index.html

University of Nebraska State Museum and
School of Biological Sciences
Harold W. Manter Laboratory of Parasitology
University of Nebraska - Lincoln
Lincoln, Nebraska
http://hwml.unl.edu/
[collections of parasites of reptiles]

URUGUAY

Museo Nacional de Historia Natural y Antropología
Zoología - Sección Herpetología
Montevideo
http://www.mec.gub.uy/munhina/mnhna.htm

VENEZUELA

Universidad Central de Venezuela
Facultad de Ciencias
Instituto de Zoología y Ecología Tropical
Museo de Biología UCV
Caracas
http://izt.ciens.ucv.ve/

Directories of Natural History Museums and Collections

Biodiversity Collections Index (GBIF)
http://www.biodiversitycollectionsindex.org/static/index.html

Herpnet
"A global network of herpetological collections data"
http://www.herpnet.org/

Natural Science Collections Alliance
http://www.nscalliance.org/

Wikipedia list of natural history museums by country
http://en.wikipedia.org/wiki/List_of_natural_history_museums

Yahoo Directory of Natural History Museums
http://dir.yahoo.com/Society_and_Culture/Environment_and_Nature/Natural_History_Museums/?b=20

APPENDIX II

Websites of Interest

MERCEDES S. FOSTER

Introduction / 345
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Introduction

In the last several decades, access to the Internet has increased enormously. It is estimated that by the end of 2010, Internet users will exceed two billion worldwide, 1.6 billion of them connecting from home, the others through public institutions (schools, libraries, etc.) or Internet cafes (ITU 2010; Miniwatts Marketing Group 2000–2010). This suggests that most individuals interested in biodiversity will have Internet access and can take advantage of a number of search engines to locate and access information about reptile biology, biodiversity, and conservation, as well as computer programs for data handling and analysis. The same can be said for sources of the permits, supplies, and equipment required for biodiversity work. Anyone seeking items for a biodiversity study will be able to access far more suppliers than we could list, particularly in their own geographic areas. Not surprisingly, most vendors listed here are located in the United States because that is the area with which we are most familiar. Only a limited number of sites that authors believe may be particularly useful are included in this appendix. Those sites are grouped into categories related to reptile biology, research, or conservation; computer programs; and equipment and supplies. Inclusion in the appendix does not constitute or imply an endorsement of any organization, vendor, product, or brand of equipment or supplies by the Editors or the by United States or other country governments.

Reptile Biology, Research, or Conservation

REPTILE INFORMATION AND ORGANIZATIONS

Center for North American Herpetology (CNAH) http://www.cnah.org/index.asp

Chelonian Research Foundation http://www.chelonian.org/

Crocodilians Natural History & Conservation http://crocodilian.com/

Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research http://www.asih.org/files/hacc-final.pdf

International Union for Conservation of Nature (IUCN) http://www.iucn.org/ A global environmental network that supports scientific research, manages field projects and brings governments, nongovernment organizations, and stakeholders together to develop and implement policy, laws and best practices.

NatureServe http://www.natureserve.org/

Species Survival Commission (IUCN/SSC) http://www.iucn.org/about/work/programmes/species/about_ssc/ A science-based network of volunteer experts deployed among Specialist Groups and Task Forces working to address conservation issues related to particular groups of organisms or topics, including the following:

- Crocodile Specialist Group http://www.iucncsg.org/ph1/modules/Home/
- Iguana Specialist Group http://www.iucn-isg.org/
- Marine Turtle Specialist Group http://iucn-mtsg.org/
- Sea Snake Specialist http://www.iucn.org/es/aib/acerca_de_la_biodiversidad/biocuriosidades/?4798/Sea-snakes
- Tortoise and Freshwater Turtle Specialist Group http://www.iucn-tftsg.org/
- Viper Specialist Group http://www.projectorianne.org/other-projects-and-initiatives/the-viper-specialist-group.html

Reptile Data Base. Peter Uetz and collaborators. http://www.reptile-database.org

Scientific and Standard English Names of Amphibians and Reptiles of North America North of Mexico. 6th ed. (updated 31 March 2011) http://www.ssarherps.org/pages/comm_names/Index.php

Turtle Conservation Fund http://www.turtleconservationfund.org

BIODIVERSITY PROGRAMS AND INFORMATION

CONABIO (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad), México http://www.conabio.gob.mx/

Discover Life in America All Taxa Biodiversity Inventory http://www.dlia.org/

Diversitas: International Programme of Biodiversity Science http://www.diversitas-international.org/?page=about_overview

Global Biodiversity Information Facility (GBIF) http://www.gbif.org/ International scientific effort to enable users throughout the world to locate and use vast quantities of already existing global biodiversity data.

Inter-American Biodiversity Information Network (IABIN) http://www.iabin.net/ A forum to foster technical collaboration and coordination among countries of the Americas in collection, sharing, and use of biodiversity information relevant to natural resources management and conservation, and education to promote sustainable development.

InBio (Instituto Nacional de Biodiversidad), Costa Rica http://www.inbio. ac.cr/en/default.html

Instituto Regional de Biodiversidad de Centroamerica (IRBIO) http://www.irbioccad.org/

United Nations Environment Programme – World Conservation Monitoring Centre (UNEP-WCMC) http://www.unep-wcmc.org

UNEP-WCMC CITES Trade Database http://www.unep-wcmc.org/citestrade/index.cfm

U.S. Fish and Wildlife Endangered Species Program http://www.fws.gov/endangered/

U.S. Geological Survey Patuxent Wildlife Research Center, *Manager's Monitoring Manual: How to Design a Wildlife Monitoring Program* http://www.pwrc.usgs.gov/monmanual/

U.S. National Park Service Inventory and Monitoring Program http://science.nature.nps.gov/im/

World Data Center for Biodiversity and Ecology http://www.nbii.gov/portal/server.pt/community/world_data_center_for_biodiversity_and_ecology/580 Provides access or linkages to online, openly accessible datasets.

TAXONOMY

EDIT (European Distributed Institute of Taxonomy) http://www.e-taxonomy.eu/

ICZN: International Commission on Zoological Nomenclature http://iczn.org/

ITIS (Integrated Taxonomic Information System) http://www.itis.gov/ An authoritative source of species names and their hierarchical classification.

PERMITS AND REGULATIONS

CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) http://www.cites.org/

IATA (International Air Transport Association) http://www.iata.org/index.htm Information on packing and shipping Dangerous Goods (HAZMAT).

U.S. Customs Service http://www.customs.gov/xp/cgov/import http://www.customs.gov/xp/cgov/export

U.S. Department of Agriculture (USDA) http://www.aphis.usda.gov

U.S. Fish and Wildlife http://permits.fws.gov

U.S. National Park Service http://science.nature.nps.gov/permits/index.html

CLIMATE DATA

U.S. Department of Commerce, including the following:

National Climate Data Center http://www.ncdc.noaa.gov/oa/ncdc.html

National Oceanic and Atmospheric Administration's (NOAA) National Weather Service http://www.weather.gov/

World Data Center for Meteorology http://www.ncdc.noaa.gov/oa/wdc/index.php

WORLDCLIM http://www.worldclim.org/ Includes environmental variables that may be useful for ecological work.

MOLECULAR DATA REPOSITORIES

DDBJ (DNA Databank of Japan) http://www.ddbj.nig.ac.jp/index-e.html

EMBL (European Molecular Biology Laboratory) Nucleotide Sequence Database http://www.ebi.ac.uk/embl/ Europe's primary nucleotide sequence resource.

EMBL EBI (European Bioinformatics Institute) http://www.ebi.ac.uk/

GenBank Database http://www.ncbi.nlm.nih.gov/genbank/ U.S. National Institutes of Health (NIH) annotated database of all publicly available DNA sequences.

MISCELLANEOUS

Centers for Disease Control and Prevention (CDC)

How to Prevent or Respond to a Snake Bite (available in multiple languages) http://www.bt.cdc.gov/disasters/snakebite.asp

Venomous Snakes and Snakebite http://www.cdc.gov/niosh/topics/snakes/#_Coral

International Field Guides Database http://www.library.illinois.edu/bix/fieldguides/main.htm

National Biological Information Infrastructure (NBII, U.S.) http://www.nbii.gov/portal/server.pt/community/nbii_home/236 A broad, collaborative program to provide increased access to data and information on the nation's biological resources. The NBII links, high-quality biological databases, information products, and analytical tools.

NBII Metadata Clearinghouse http://metadata.nbii.gov/clearinghouse/ Searchable collection of standardized metadata-based descriptions of biological data sets and information products.

NBII Metadata Standards http://www.nbii.gov/portal/server.pt/community/metadata/704

Tree of Life http://tolweb.org/tree/

U.S. Geological Survey Biological Informatics Program http://biology.usgs.gov/bio/

Computer Software

ArcGis: GIS and Mapping Software System (Esri) http://www.esri.com/products/index.html

I^{3}S: Interactive Individual Identification System http://www.reijns.com/i3s/index.html

Palm Infocenter http://www.palminfocenter.com/

Patuxent Wildlife Research Center (PWRC) Software Archive http://www.mbr-pwrc.usgs.gov/software.html

Programs:

ALEX	http://www.ecology.uq.edu.au/index.html
Biota	http://viceroy.eeb.uconn.edu/Biota2Pages/about_biota.html
CAPTURE	http://www.mbr-pwrc.usgs.gov/software/capture.html
DENSITY	http://www.landcareresearch.co.nz/services/software/density
DISTANCE	http://www.ruwpa.st-and.ac.uk/distance/

EstimateS	http://viceroy.eeb.uconn.edu/estimates
MARK	http://warnercnr.colostate.edu/~gwhite/mark/mark.htm
PRESENCE	http://www.proteus.co.nz/software.html (see also PWRC Software Archive)
RAMAS	http://www.ramas.com
RELEASE	http://www.mbr-pwrc.usgs.gov/software/release.shtml
VORTEX	http://www.vortex9.org/vortex.html

Vendors of Equipment and Supplies

GENERAL PRODUCTS FOR FIELD RESEARCH

Ben Meadows http://benmeadows.com

BioQuip http://www.bioquip.com/

Cabela's http://www.cabelas.com/

Carolina Biological Supply http://www.carolina.com/

Forestry Suppliers http://www.forestry-suppliers.com/

Ward's Natural Science http://wardsci.com/

CAPTURE EQUIPMENT

Midwest Tongs http://tongs.com/ [handling and other equipment]

Memphis Net and Twine http://www.memphisnet.net/ [nets, traps]

Nichols Net and Twine Company http://www.nicholsnetandtwine.com/ [nets, seines, traps; standard and custom-made]

Sterling Net and Twine - Marine Products http://www.sterlingnets.com/marine.html [minnow traps, nets, seines, custom-made nets]

Target Zone Sports http://www.blow-gun.com/ [stun darts]

DATALOGGERS, SENSORS, AND WEATHER MONITORING SYSTEMS

Campbell Scientific http://www.campbellsci.com/index.cfm [also digital cameras with triggering systems]

Climatronics http://www.climatronics.com

Delta-T Devices http://www.delta-t.co.uk

Maxim http://www.maxim-ic.com/products/ibutton [ibuttons]

Omega Engineering http://www.omega.com

Onset Computer Corporation http://www.onsetcomp.com

Skye Instruments http://www.skyeinstruments.com

MARKING AND IDENTIFICATION

Allen-Bailey Tag and Label http://www.abtl.com/

AVID MicroChip I.D. http://www.avidmicrochip.com [passive integrated transponders]

Fish Eagle Trading http://www.psmfc.org/pittag/Software_and_ Documentation/pitvendors.html

Home Spy http://www.homespy.com/powder.htm [fluorescent marking materials]

National Band and Tag http://www.nationalband.com/

Radiant Color http://www.radiantcolor.com/ [fluorescent pigments]

UNO bv http://www.unobv.com/1/Home.html [transponders and scanners]

Valley Vet http://www.valleyvet.com/ [small animal tattooing equipment]

RADIOTELEMETRY

Biotelemetry Equipment Manufacturers Directory http://www.biotelem.org/manufact.htm

ATS Advanced Telemetry Systems http://www.atstrack.com/

AVM Instrument Company http://www.avminstrument.com/

Holohil Systems http://www.holohil.com

Telonics http://www.telonics.com/

Wildlife Materials http://wildlifematerials.com/

MISCELLANEOUS FIELD EQUIPMENT

Biologika Group http://www.biologikagroup.com/

Fuhrman Diversified http://www.fieldcam.com [field camera systems; other reptile equipment]

Go-Devil Manufacturers of Louisiana http://www.godevil.com/ [surface-drive engines]

Miller and Weber http://www.millerweber.com [quick-reading thermometers]

Pendragon Forms http://www.pendragonsoftware.com/ [digital data forms]

Preservation Equipment http://www.preservationequipment.com/ [Resistall paper]

Sandpiper Technologies http://www.sandpipertech.com/info.html [wildlife research equipment, including video probe systems]

StealthCam http://www.stealthcam.net/ [field camera systems]

CHEMICALS AND DRUGS

AstraZeneca http://www.astrazeneca.com/Home

Fisher Scientific International http://www.fishersci.com

Pfizer Animal Health http://www.pfizeranimalhealth.com/default.aspx#

Schering-Plough Animal Health http://www.intervet.com/ http://www.intervetusa.com/

Sigma-Aldrich http://www.sigmaaldrich.com

MOLECULAR ANALYSIS

Amresco http://www.amresco-inc.com

EuroCone http://www.euroclone.net [cryogenics]

Nalge Nunc International http://www.nalgenunc.com

PGC Scientifics http://www.pgcscientifics.com/

Promega https://www.promega.com/

Qiagen http://www.qiagen.com

Taylor-Wharton http://www.taylorwharton.com/default.aspx [manufactures cryogenic refrigerators (liquid nitrogen tanks); contact for vendor information]

VWR Scientific http://www.vwrsp.com

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LITERATURE CITED

Abate, T. 1992. Environmental rapid-assessment programs have appeal and critics. BioScience 42:486–489.

Abercrombie, III, C. L. 1989. Population dynamics of the American alligator. Pp. 1–16. In *Crocodiles: Their Ecology, Management, and Conservation*. Special Publication of the Crocodile Specialist Group of the Species Survival Commission of the International Union for Conservation of Nature and Natural Resources, Gland, Switzerland.

Abrahamson, W. G., and D. C. Hartnett. 1990. Pine flatwoods and dry prairies. Pp. 103–149. In R. L. Myers and J. J. Ewel (eds.), *Ecosystems of Florida*. University of Central Florida Press, Orlando, Florida.

Abuys, A. 2003. *De Slangen van Suriname en de andere Guyana's*. Gopher, Groningen, The Netherlands.

Adalsteinsson, S. A., W. R. Branch, S. Trape, L. J. Vitt, and S. B. Hedges. 2009. Molecular phylogeny, classification, and biogeography of snakes of the Family Leptotyphlopidae (Reptilia, Squamata). Zootaxa 2244:1–50.

Adams, C. E., and J. K. Thomas. 2008. *Texas Rattlesnake Roundups*. Texas A&M University Press, College Station, Texas.

Adams, M. J., K. O. Richter, and W. P. Leonard. 1997. Surveying and monitoring amphibians using aquatic funnel traps. Pp. 47–54. In D. H. Olson, W. P. Leonard, and R. B. Bury (eds.), *Sampling Amphibians in Lentic Habitats: Methods and Approaches for the Pacific Northwest*. Society for Northwestern Vertebrate Biology, Northwest Fauna 4.

Adams, M. J., S. D. West, and L. Kalmbach. 1999. Amphibian and reptile surveys of U.S. Navy lands on the Kitsap and Toandos Peninsulas, Washington. Northwestern Naturalist 80:1–7.

Adest, G. A., J. Jarchow, and B. Brydolf. 1988. A method for manual ventilation of tranquilized tortoises. Herpetological Review 19:80.

Agresti, A. 1990. *Categorical Data Analysis*. Wiley, New York.

Akin, J. A. 1998. Fourier series estimation of ground skink population density. Copeia 1998:519–522.

Akre, T. S. B. 2002. Growth, Maturity, and Reproduction of the Wood Turtle, *Clemmys insculpta* (Le Conte, 1830) in Virginia. Unpubl. Ph.D. dissert., George Mason University, Fairfax, Virginia.

Alcala, A. C. 1986. *Guide to Philippine Flora and Fauna*. Vol. X. *Amphibians and Reptiles*. Natural Resources Management Center, Ministry of Natural Resources and University of the Philippines. Manila, Philippines.

Alcala, A. C., and W. C. Brown. 1967. Population ecology of the tropical scincoid lizard, *Emoia atrocostata,* in the Philippines. Copeia 1967:596–604.

Aldridge, R. D. 1979. Seasonal spermatogenesis in sympatric *Crotalus viridis* and *Arizona elegans* in New Mexico. Journal of Herpetology 13:187–192.

Aldridge, R. D. 2001. Reproductive anatomy, mating season and cost of reproduction in the Glossy Snake (*Arizona elegans*). Amphibia-Reptilia 22:243–249.

Aldridge, R. D., and W. S. Brown. 1995. Male reproductive cycle, age at maturity, and cost of reproduction in the Timber Rattlesnake (*Crotalus horridus*). Journal of Herpetology 29:399–407.

Allanson, M., and A. Georges. 1999. Diet of *Elseya purvisi* and *Elseya georgesi* (Testudines: Chelidae), a sibling species pair of freshwater turtles from eastern Australia. Chelonian Conservation and Biology 3:473–476.

Allison, A., D. Bickford, S. Richards, and G. Torr. 1998. Herpetofauna. Pp. 58–62. In A. L. Mack (ed.), *A Biological Assessment of the Lakekamu Basin, Papua New Guinea*. Rapid Assessment Program Working Papers 9, Conservation International, Washington, DC.

Allison, G. W., J. Lubchenko, and M. H. Carr. 1998. Marine reserves are necessary but not sufficient for marine conservation. Ecological Applications 8 (suppl.):S79–S92.

Allsteadt, J., and J. W. Lang. 1995. Sexual dimorphism in the genital morphology of young American Alligators, *Alligator mississipiensis*. Herpetologica 51:314–325.

Almli, L. M., and G. M. Burghardt. 2006. Environmental enrichment alters the behavioral profile of ratsnakes (*Elaphe*). Journal of Applied Animal Welfare Science 9:85–109.

Altimari, W. 1998. Venomous snakes: A safety guide for reptile keepers. Society for the Study of Amphibians and Reptiles, Herpetological Circular 26:1–24.

Alvarez del Toro, M. 1960. Reptiles de Chiapas. Instituto Zoológico del Estado, Tuxtla Gutiérrez, Chiapas, México.

Amaral, A. do. 1977. *Brazilian Snakes: A Color Iconography*. Melhoramentos, Editora da Universidade de São Paulo, São Paulo, Brazil.

Ananjeva, N. 1997. Diversity of agamids (Sauria, Reptilia): Ecological forms and morphology of skin receptors. Pp. 297–304. In H. Ulrich (ed.), *Tropical Biodiversity and Systematics. Proceedings of the International Symposium on Biodiversity and Systematics in Tropical Ecosystems, Bonn, 1994*. Zoologisches Forshungsinstitut und Museum Alexander Koenig.

Ananjeva, N. B., Kh. Munkhbayar, N. L. Orlov, V. F. Orlova, D. V. Semenov, and Kh. Terbish (eds.). 1997. *Zem novodnye i presmykayushchiesya Mongolii. Presmykayushchiesya*. [*Amphibians and Reptiles of Mongolia*. Vol. *Reptiles*] In V. E. Sokolov (ed.), *Vertebrates of Mongolia*. KMK Scientific, Moscow, Russia.

Anderson, D. R. 2003. Response to Engeman: Index values rarely constitute reliable information. Wildlife Society Bulletin 31:288–291.

Anderson, D. R., K. P. Burnham, B. C. Lubow, L. Thomas, P. S. Corn, P. A. Medica, and R. W. Marlow. 2001. Field trials of line transect methods applied to estimation of Desert Tortoise abundance. Journal of Wildlife Management 65:583–597.

Anderson, D. R., K. P. Burnham, G. C. White, and D. L. Otis. 1983. Density estimation of small-mammal populations using a trapping web and distance sampling methods. Ecology 64:674–680.

Anderson, D. R., J. L. Laake, B. R. Crain, and K. P. Burnham. 1979. Guidelines for line transect sampling of biological populations. Journal of Wildlife Management 43:70–78.

Anderson, N. L., B. Coupe, G. Perry, T. E. Hetherington, and J. B. Williams. 2000. Field use of propofol: A rapid recovery anesthetic with data from Brown Treesnakes (*Boiga irregularis*). Herpetological Review 31:161–163.

Anderson, P. K. 1961. Variation in populations of Brown Snakes, genus *Storeria*, bordering the Gulf of Mexico. American Midland Naturalist 66:235–249.

Anderson, R. M. 1965. Methods of collecting and preserving vertebrate animals. 4th ed., rev. National Museum of Canada (Biological Series 18) Bulletin 69:1–141.

Anderson, S. C. 1999. *The Lizards of Iran*. Contributions to Herpetology Vol. 15. Society for the Study of Amphibians and Reptiles, Ithaca, New York.

Andrews, K. M., and J. W. Gibbons. 2005. How do highways influence snake movement? Behavioral responses to roads and vehicles. Copeia 2005:772–782.

Andrews, R. M., and J. A. Stamps. 1994. Temporal variation in sexual size dimorphism of *Anolis limifrons* in Panama. Copeia 1994:613–622.

Andrews, R. M., and J. S. Wright. 1994. Long-term population fluctuations of a tropical lizard: A test of causality. Pp. 267–285. In L. J. Vitt and E. R. Pianka (eds.), *Lizard Ecology: Historical and Experimental Perspectives.* Princeton University Press, Princeton, New Jersey.

Angilletta, M. J., Jr., and A. R. Krochmal. 2003. The thermochron: A truly miniature and inexpensive temperature-logger. Herpetological Review 34:31–32.

Animal Behavior Society and Association for the Study of Animal Behaviour. 2002. Guidelines for the treatment of animals in behavioural research and teaching. Animal Behaviour 63:195–199.

Antworth, R. L., D. A. Pike, and E. E. Stevens. 2005. Hit and run: Effects of scavenging on estimates of roadkilled vertebrates. Southeastern Naturalist 4:647–656.

Applied Biomathematics. 2007. *RAMAS*. Setauket, New York. http://www.ramas.com

Arena, P. C., K. C. Richardson, and L. K. Cullen. 1988. Anaesthesia in two species of large Australian skink. Veterinary Record 123:155–158.

Arnold, E. N. 1984. Variation in the cloacal and hemipenial muscles of lizards and its bearing on their relationships. Pp. 47–85. In M. W. J. Ferguson (ed.), *The Structure, Development and Evolution of Reptiles.* Symposium of the Zoological Society of London no. 52.

Arnold, E. N. 1986. Mite pockets of lizards, a possible means of reducing damage by ectoparasites. Biological Journal of the Linnean Society 29:1–21.

Arnold, E. N. 1988. Caudal autotomy as a defense. Pp. 235–273. In C. Gans and R. B. Huey (eds.), *Biology of the Reptilia*, Vol. 16. *Ecology B Defense and Life History.* Alan R. Liss, New York.

Arnold, E. N. 2002. *Reptiles and Amphibians of Europe*. Princeton University Press, Princeton, New Jersey.

Artz, M. C. 1989. Impacts of linear corridors on perennial vegetation in the east Mojave Desert: Implications for environmental management and planning. Natural Areas Journal 9:117–129.

Ash, A. N. 1997. Disappearance and return of plethodontid salamanders to clearcut plots in the southern Blue Ridge Mountains. Conservation Biology 11:983–989.

Ashley, E. P., and J. T. Robinson. 1996. Road mortality of amphibians, reptiles and other wildlife on the Long Point Causeway, Lake Erie, Ontario. Canadian Field-Naturalist 110:403–412.

Ashley, L. M. 1962. *Laboratory Anatomy of the Turtle*. W. C. Brown, Dubuque, Iowa.

ASIH (American Society of Ichthyologists and Herpetologists). 2004. *Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research.* 2nd ed. http://www.asih.org/files/hacc-final.pdf.

Asma, S. T. 2001. *Stuffed Animals and Pickled Heads. The Culture and Evolution of Natural History Museums.* Oxford University Press, New York.

Atkinson, A. J., R. N. Fisher, C. J. Rochester, and C. W. Brown. 2003. *Sampling Design Optimization and Establishment of Baselines for Herpetofauna Arrays at the Point Loma Ecological Reserve.* U.S. Geological Survey Western Ecological Research Center Technical Report prepared for Cabrillo National Monument, National Park Service. USGS/WERC, Sacramento, California.

Auerbach, R., L. Kubai, D. Knighton, and J. Folkman. 1974. A simple procedure for the long-term cultivation of chicken embryos. Developmental Biology 41:391–394.

Auffenberg, W. 1981. Behavior of *Lissemys punctata* (Reptilia, Testudinata, Trionychidae) in a drying lake in Rajasthan, India. Journal of the Bombay Natural History Society 78:487–493.

August, P., C. Baker, C. LaBash, and C. Smith. 1996. The geographic information system for storage and analysis of biodiversity data. Pp. 235–246. In D. E. Wilson, F. R. Cole, J. D. Nichols, R. Rudran, and M. S. Foster (eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Mammals.* Smithsonian Institution Press, Washington, DC.

Auliya, M. A., and W. Erdelen. 1999. A field study of the water monitor lizard (*Varanus salvator*) in West Kalimantan, Indonesia: New methods and old problems. Pp. 247–266. In H.-G. Horn and W. Böhme (eds.), *Advances in Monitor Research II.* Mertensiella 11.

Austin, M. P., and P. C. Heyligers. 1991. New approaches to vegetation survey design: Gradsect sampling. Pp. 31–36. In C. R. Margules and M. P. Austin (eds.), *Nature Conservation: Cost-Effective Biological Surveys and Data Analysis.* CSIRO, Melbourne, Australia.

Auth, D. L. 1975. Behavioral ecology of basking in the Yellow-bellied Turtle, *Chrysemys scripta scripta* (Schoepff). Bulletin of the Florida State Museum, Biological Science 20:1–45.

Avila-Pires, T. C. S. 1995. *Lizards of Brazilian Amazonia (Reptilia: Squamata).* Zoologische Verhandelingen, Nationaal Natuurhistorisch Museum (Leiden) 299:1–706.

AVMA (American Veterinary Medical Association). 2007. *AVMA Guidelines on Euthanasia.* http://www.avma.org/issues/animal_welfare/euthanasia.pdf

Axtell, R. W. 1988a. *Interpretive Atlas of Texas Lizards.* No. 4. *Sceloporus variabilis*. 11 pp. + map #3. Privately published [available from the author, raxtell@siue.edu].

Axtell, R. W. 1988b. *Interpretive Atlas of Texas Lizards.* No. 5. *Sceloporus graciosus*. 4 pp. + map #3. Privately published [available from the author, raxtell@siue.edu].

Axtell, R. W. 1992. *Interpretive Atlas of Texas Lizards.* No. 12. *Sceloporus magister.* 8 pp. + map #7. Privately published [available from the author, raxtell@siue.edu].

Axtell, R. W. 1996. *Interpretive Atlas of Texas Lizards.* No. 16. *Phrynosoma cornutum* (Harlan). 52 pp. + map #9. Privately published [available from the author, raxtell@siue.edu].

Axtell, R. W. 1999. *Interpretive Atlas of Texas Lizards.* No. 22. *Eumeces septentrionalis* (Baird). 14 pp. + map #12. Privately published [available from the author, raxtell@siue.edu].

Axtell, R. W. 2000. *Interpretive Atlas of Texas Lizards.* No. 23. *Eumeces multivirgatus* (Hallowell). 8 pp. + map 12. Privately published [available from the author, raxtell@siue.edu].

Axtell, R. W. 2003a. *Interpretive Atlas of Texas Lizards.* No. 27. *Scincella lateralis*. 41 pp. + map 13. Privately published [available from the author, raxtell@siue.edu].

Axtell, R. W. 2003b. *Interpretive Atlas of Texas Lizards.* No. 28. *Cnemidophorus exsanguis*. 9 pp. + map 13. Privately published [available from the author, raxtell@siue.edu].

Ayarzagüena, J. 1983. Ecologia del caiman de anteojos o baba (*Caiman crocodilus* L.) en los Llanos de Apure (Venezuela). Doñana Acta Vertebrata 10:1–136.

Azuma, D. L., J. A. Baldwin, and B. R. Noon. 1990. *Estimating the Occupancy of Spotted Owl Habitat Areas by Sampling and Adjusting for Bias.* USDA General Technical Report PSW-124, Pacific Southwest Research Station, Forest Service, Berkeley, California.

Baha El Din, S. 2006. *A Guide to the Reptiles and Amphibians of Egypt.* American University in Cairo Press, Cairo, Egypt.

Bailey, L. L., T. R. Simons, and K. H. Pollock. 2004a. Estimating detection probability parameters for *Plethodon* salamanders using the robust capture-recapture design. Journal of Wildlife Management 68:1–13.

Bailey, L. L., T. R. Simons, and K. H. Pollock. 2004b. Spatial and temporal variation in detection probability of *Plethodon* salamanders using the robust capture-recapture design. Journal of Wildlife Management 68:14–24.

Bailey, T. C., and A. C. Gatrell. 1995. *Interactive Spatial Data Analysis.* Longman Scientific Technical, Harlow, Essex, England.

Baird, S. F. 1859. [1858]. Description of new genera and species of North American lizards in the museum of the Smithsonian Institution. *Proceedings of the Academy of Natural Sciences of Philadelphia* 10:253–256.

Baker, B. W. 2008. A brief overview of forensic herpetology. Applied Herpetology 5:307–318.

Bakken, G. S. 1992. Measurement and application of operative and standard operative temperatures in ecology. American Zoologist 32:194–216.

Bakken, G. S., and D. M. Gates. 1975. Heat-transfer analysis of animals: Some implications for field ecology, physiology, and evolution. Pp. 255–290. In D. M. Gates and R. B. Schmerl (eds.), *Perspectives of Biophysical Ecology*. Springer, New York.

Balazs, G. H. 1999. Factors to consider in the tagging of sea turtles. Pp. 101–109. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, and M. Donnelly (eds.), *Research and Management Techniques for the Conservation of Sea Turtles*. IUCN/SSC Marine Turtle Specialist Group Publication no. 4.

Balazs, G. H., R. K. Miya, and S. C. Beavers. 1996. Procedures to attach a satellite transmitter to the carapace of an adult green turtle, *Chelonia mydas*. Pp. 21–26. In J. A. Keinath, D. E. Barnard, J. A. Musick., and B. A. Bell (compilers), *Proceedings of the Fifteenth Annual Workshop on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFSC-387. National Marine Fisheries Service, Miami, Florida.

Balgooyen, T. G. 1977. Collecting methods for amphibians and reptiles. US Bureau of Land Management Technical Note 299:1–12.

Bannikov, A. G. 1958. Materials on the fauna and biology of amphibians and reptiles of Mongolia. Bulletin Moscow Academy of Sciences, Biology 63:71–91. [Bulleten Moskovskoe obshchestvo ispytateleï prirody, Otdel Bologicheskii; in Russian with English summary]

Banta, B. H. 1957. A simple trap for collecting desert reptiles. Herpetologica 13:174–176.

Banta, B. H. 1962. A preliminary account of the herpetofauna of the Saline Valley hydrographic basin, Inyo County, California. Wasmann Journal of Biology 20:161–251.

Barbault, R. 1967. Recherches écologiques dans la savane de Lamto (Côte d'Ivoire): Le cycle annuel de la biomasse des amphibiens et des lézards. Terre et Vie, Revue D'Écologie Appliquée 21:297–318.

Barbault, R. 1976. Population dynamics and reproductive patterns of three African skinks. Copeia 1976:483–490.

Barker, M. A., and D. D. Hobson. 1996. Artificial refuges with transects as a possible reptile survey methodology. British Herpetological Society Bulletin 55:8–14.

Barlow, J. 1999. Trackline detection probability for long-diving whales. Pp. 209–221. In G. W. Garner, S. C. Amstrup, J. L. Laake, B. F. J. Manly, L. L. McDonald, and D. G. Robertson (eds.), *Marine Mammal Survey and Assessment Methods*. Balkema, Rotterdam, The Netherlands.

Barlow, J., C. W. Oliver, T. D. Jackson, and B. L. Taylor. 1988. Harbor Porpoise, *Phocoena phocoena,* abundance estimation for California, Oregon, and Washington: II. Aerial surveys. Fishery Bulletin 86:433–444.

Barter, L. S., M. G. Hawkins, R. J. Brosnan, J. F. Antognini, and B. H. Pypendop. 2006. Median effective dose of isoflurane, sevoflurane, and desflurane in green iguanas. American Journal of Veterinary Research 67:392–397.

Bas López, S. 1982. La comunidad herpetológica de Caurel: Biogeografía y ecología. Amphibia-Reptilia 3:1–26.

Bates, H. W. 1863. *The Naturalist on the River Amazons*. John Murray, London, England.

Bauer, A. M. 1999. Evolutionary scenarios in the *Pachydactylus* group geckos of southern Africa: New hypotheses. African Journal of Herpetology 48:53–62.

Bauer, A. M., and R. A. Sadlier. 1992. The use of mouse glue traps to capture lizards. Herpetological Review 23:112–113.

Bauer, A. M., and R. A. Sadlier. 2000. *The Herpetofauna of New Caledonia*. Contributions to Herpetology Vol. 17. Society for the Study of Amphibians and Reptiles, Ithaca, New York.

Bayliss, P. 1986. Factors affecting aerial surveys of marine fauna, and their relationship to a census of dugongs in the coastal waters of the Northern Territory. Australian Wildlife Research 13:27–37.

Bayliss, P. 1987. Survey methods and monitoring within crocodile management programmes. Pp. 157–175. In G. J. W. Webb, S. C. Manolis, and P. J. Whitehead (eds.), *Wildlife Management: Crocodiles and Alligators*. Surrey Beatty, Chipping Norton, and the Conservation Commission of the Northern Territory, New South Wales, Australia.

Beauchamp B., B. Wone, S. Bros, and M. Kutilek. 1998. Habitat use of the Flat-tailed Horned Lizard (*Phrynosoma mcallii*) in a disturbed environment. Journal of Herpetology 32:210–216.

Beaupre, S. J. 1995. Comparative ecology of the Mottled Rock Rattlesnake, *Crotalus lepidus*, in Big Bend National Park. Herpetologica 51:45–56.

Beaupre, S. J., and R. W. Beaupre. 1994. An inexpensive data collection system for temperature telemetry. Herpetologica 50:509–516.

Beaupre, S. J., and D. J. Duvall. 1998. Integrative biology of rattlesnakes: Contributions to biology and evolution. BioScience 48:531–538.

Beavers, S. C., and E. R. Cassano. 1996. Movements and dive behavior of a male sea turtle (*Lepidochelys olivacea*) in the eastern tropical Pacific. Journal of Herpetology 30:97–104.

Beavers, S. C., and F. L. Ramsey. 1998. Detectability analysis in transect surveys. Journal of Wildlife Management 62:948–957.

Bedford, G. S., K. Christian, and B. Barrette. 1995. A method for catching lizards in trees and rock crevices. Herpetological Review 26:21–22.

Beggs, K., J. Young, A. Georges, and P. West. 2000. Ageing the eggs and embryos of the Pig-nosed Turtle, *Carettochelys insculpta* (Chelonia: Carettochelyidae), from northern Australia. Canadian Journal of Zoology 78:373–392.

Begon, M., J. L. Harper, and C. R. Townsend. 1990. *Ecology: Individuals, Populations, and Communities*. 2nd ed. Blackwell Science, Cambridge, Massachusetts.

Beissinger, S. R., and M. I. Westphal. 1998. On the use of demographic models of population viability in endangered species management. Journal of Wildlife Management 62:821–841.

Bekoff, M. (ed). 1998. *Encyclopedia of Animal Rights and Animal Welfare*. 2nd ed. Greenwood, Santa Barbara, California.

Bellini, C., M. H. Godfrey, and T. M. Sanches. 2001. Metal tag loss in wild juvenile Hawksbill Sea Turtles (*Eretmochelys imbricata*). Herpetological Review 32:172–174.

Bennett, D. 1999. *Expedition Field Technique: Reptiles and Amphibians*. Geography Outdoors, Royal Geographical Society, London, England.

Bennett, D., and K. Hampson. 2003. Further observations of *Varanus olivaceus* on Polillo. In K. Hampson, D. Bennett, P. Alviola, T. Clements, C. Galley, M. V. Hilario, M. Ledesma, M. A. Manuba, A. Pulumbarit, A. Reyes, E. Rico, and S. Walker. 2003. *Wildlife and Conservation in the Polillo Islands, Philippines*: *Polillo Project Final Report; Species Guide*. Multimedia CD (ISBN 1-904589-00-6) Viper Press, Glossop, Great Britain.

Bennett, D., K. Hampson, and V. Yngente. 2001. A noose trap for catching a large arboreal lizard, *Varanus olivaceus*. Herpetological Review 32:167–168.

Bennett, D. H., J. W. Gibbons, and J. C. Franson. 1970. Terrestrial activity in aquatic turtles. Ecology 51:738–740.

Bennett, R. A. 1996. Anesthesia. Pp. 241–247. In D. R. Mader (ed.), *Reptile Medicine and Surgery*. Saunders, Philadelphia, Pennsylvania.

Bennett, R. A. 1998. Pain and analgesia in reptiles and amphibians. *Association of Reptilian and Amphibian Veterinarians Annual Proceedings* 1998:1–5.

Bennett, R. A., J. Schumacher, K. Hedjazi-Haring, and S. M. Newell. 1998. Cardiopulmonary and anesthetic effects of propofol administered intraosseously to green iguanas. Journal of the American Veterinary Medical Association 212:93–98.

Berger, D. D., and H. C. Mueller. 1959. The bal-chatri: A trap for the birds of prey. Bird-Banding 30:18–26.

Berger, W. H., and F. L. Parker. 1970. Diversity of planktonic Foraminifera in deep-sea sediments. Science 168:1345–1347.

Berlin, G. L. L., and T. E. Avery. 2003. *Fundamentals of Remote Sensing and Airphoto Interpretation*. 6th ed. Prentice Hall, Upper Saddle River, New Jersey.

Bernardino, F. S., Jr., and G. H. Dalrymple. 1992. Seasonal activity and road mortality of the snakes of the Pa-hay-okee wetlands of Everglades National Park, USA. Biological Conservation 62:71–75.

Bertelsen, M. F., C. Mosley, G. J. Crawshaw, D. Dyson, and D. A. Smith. 2005. Inhalation anesthesia in Dumeril's Monitor (*Varanus dumerili*) with isoflurane, sevoflurane, and nitrous oxide: Effects of inspired gases on induction and recovery. Journal of Zoo and Wildlife Medicine 36:62–68.

Bertona, M., and M. Chiaraviglio. 2003. Reproductive biology, mating aggregations, and sexual dimorphism of the Argentine Boa

Constrictor (*Boa constrictor occidentalis*). Journal of Herpetology 37:510–516.

Bertram, N., and K. W. Larsen. 2004. Putting the squeeze on venomous snakes: Accuracy and precision of length measurements taken with the "squeeze box." Herpetological Review 35:235–238.

Bertrand, M., and I. Ineich. 1986. Sur deux nouvelles espèces de Pterygosomatidae ectoparasites de Gekkonidae. Relations entre les distributions de l'hôte et du parasite. Acarologia 27:141–149.

Bertrand, M., and I. Ineich. 1987. Contribution à la connaissance des Pterygosomatidae du Pacifique Sud. Acarologia 28:241–250.

Bertrand, M., and I. Ineich. 1989. Répartition des Pterygosomatidae du genre *Geckobia* Megnin, 1878 ectoparasites du gecko *Gehyra oceanica* (Lesson, 1826), en Polynésie Française. Acarologia 30:365–371.

Besnard, A., M. Cheylan, T. Couturier, A. Bertolero, and A. Astruc. 2007. Test of a new method for Hermann's Tortoise population monitoring. *14th European Congress of Herpetology and Societas Europaea Herpetologica 14th Ordinary General Meeting, 19–23 September 2007, Porto, Portugal: Programme & Abstracts,* p. 182.

Bezy, R. L. 1988. The natural history of the night lizards, family Xantusiidae. Pp. 1–12. In H. F. De Lisle, P. R. Brown, B. Kaufman, and B. M. McGurty (eds.), *Proceedings of the Conference on California Herpetology*. Southwestern Herpetologists Society Special Publication no. 4, Sunlight Press, Lakeside, California.

Bider, J. R., and W. Hoek. 1971. An efficient and apparently unbiased sampling technique for population studies of Painted Turtles. Herpetologica 27:481–484.

Billett, F., C. Gans, and P. F. A. Maderson. 1985. Why study reptilian development? Pp. 1–39. In C. Gans, G. Billett, and P. F. A. Maderson (eds.), *Biology of the Reptilia,* Vol. 14. *Development A*. Wiley, New York. .

Bishop, C. A., R. J. Brooks, J. H. Carey, P. Ng, R. J. Norstrom, and D. R. S. Lean. 1991. The case for a cause-effect linkage between environmental contamination and development in eggs of the Common Snapping Turtle (*Chelydra s. serpentina*) from Ontario, Canada. Journal of Toxicology and Environmental Health 33:521–547.

Bishop, J. E. 1959. A histological and histochemical study of the kidney tubule of the Common Garter Snake, *Thamnophis sirtalis,* with special reference to the sexual segment in the male. Journal of Morphology 104:307–357.

Bishop, S. C., and W. J. Schoonmacher. 1921. Turtle hunting in midwinter. Copeia 96:37–38.

Bjorndal, K. A. 1999. Priorities for research in foraging habitats. Pp. 12–14. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, and M. Donnelly (eds.), *Research and Management Techniques for the Conservation of Sea Turtles*. IUCN/SSC Marine Turtle Specialist Group Publication no. 4.

Bjorndal, K. A., A. B. Bolten, R. A. Bennett, E. R. Jacobson, T. J. Wronski, J. J. Valeski, and P. J. Eliazar. 1998. Age and growth in sea turtles: Limitations of skeletochronology for demographic studies. Copeia 1998:23–30.

Blair, W. F. 1957. Changes in vertebrate populations under conditions of drought. Pp. 273–275. In Cold Spring Harbor Symposia on Quantitative Biology Vol. 22, *Population Studies: Animal Ecology and Demography.* Cold Spring Harbor, New York.

Blanc, F. 1974. Table de développement de *Chamaeleo lateralis* Gray, 1831. Annales d'Embryologie et de Morphogenèse 7:99–115.

Blanchard, F. N. 1921. A revision of the King Snakes: Genus *Lampropeltis*. US National Museum Bulletin 114:1–260. Washington, DC.

Blanchard, F. N., and E. B. Finster. 1933. A method of marking living snakes for future recognition, with a discussion of some problems and results. Ecology 14:334–347.

Blankenship, E. L., T. W. Bryan, and S. P. Jacobsen. 1990. A method for tracking tortoises using fluorescent powder. Herpetological Review 21:88–89.

Blouin-Demers, G. 2003. Precision and accuracy of body-size measurements in a constricting, large-bodied snake (*Elaphe obsoleta*). Herpetological Review 34:320–323.

Blouin-Demers, G., K. A. Prior, and P. J. Weatherhead. 2002. Comparative demography of Black Rat Snakes (*Elaphe obsoleta*) in Ontario and Maryland. Journal of Zoology, London 256:1–10.

Boarman, W. I. 2003. Managing a subsidized predator population: Reducing Common Raven predation on Desert Tortoises. Environmental Management 32:205–217.

Boarman, W. I., M. L. Beigel, G. C. Goodlett, and M. Sazaki. 1998. A passive integrated transponder system for tracking animal movements. Wildlife Society Bulletin 26:886–891.

Boarman, W. I., T. Goodlett, G. Goodlett, and P. Hamilton. 1998. Review of radio transmitter attachment techniques for turtle research and recommendations for improvement. Herpetological Review 29:26–33.

Boeadi, R. Shine, A. M. Sugardijto, M. H. Sinaga. 1998. Biology of the commercially-harvested ratsnake (*Ptyas mucosus*) and cobra (*Naja sputatrix*) in central Java. Mertensiella 9:99–104.

Boglioli, M. D., C. Guyer, and W. K. Michener. 2003. Mating opportunities of female Gopher Tortoises, *Gopherus polyphemus,* in relation to spatial isolation of females and their burrows. Copeia 2003:846–850.

Böhme, W. 1995. Hemiclitoris discovered: A fully differentiated erectile structure in female monitor lizards (*Varanus* spp.) (Reptilia: Varanidae). Journal of Zoological Systematics and Evolutionary Research 33:129–132.

Bolten, A. B., K. A. Bjorndal, J. S. Grumbles, and D. W. Owens. 1992. Sex ratio and sex-specific growth rates of immature green turtles, *Chelonia mydas,* in the southern Bahamas. Copeia 1992:1098–1103.

Bonin, F., B. Devaux, and A. Dupré. 2006. *Turtles of the World*. Johns Hopkins University Press, Baltimore, Maryland.

Bonnet, X., and G. Naulleau. 1996. Catchability in snakes: Consequences for estimates of breeding frequency. Canadian Journal of Zoology 74:233–239.

Bonnet, X., G. Naulleau, and R. Shine. 1999. The dangers of leaving home: Dispersal and mortality in snakes. Biological Conservation 89:39–50.

Bons, J., and P. Geniez. 1996. *Amphibiens et Reptiles du Maroc (Sahara Occidental compris). Atlas Biogéographique*. Asociación Herpetológica Española, Barcelona, Spain.

Booker, W. C., and L. M. Ehrhart. 1989. Aerial surveys of marine turtle carcasses in National Marine Fisheries Service Statistical Zones 28 and 29; 11 August 1987 to 31 December 1988. Pp. 15–18. In S. A. Eckert, K. L. Eckert, and T. H. Richardson (compilers), *Proceedings of the Ninth Annual Workshop on Sea Turtle Conservation and Biology.* NOAA Technical Memorandum NMFS-SEFC-232. National Marine Fisheries Service, Southeast Fisheries Center, Miami, Florida.

Booker, W. C., and L. M. Ehrhart. 1990. Aerial surveys of marine turtle carcasses in National Marine Fisheries Service Statistical Zones 28, 29, and 30; 11 August 1987 to 31 December 1989. Pp. 99–102. In T. H. Richardson, J. I. Richardson, and M. Donnelly (compilers), *Proceedings of the Tenth Annual Workshop on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFC-278. National Marine Fisheries Service, Miami, Florida.

Boone, J. L., and E. A. Larue. 1999. Effects of marking *Uta stansburiana* (Sauria: Phrynosomatidae) with xylene-based paint. Herpetological Review 30:33–34.

Borchers, D. L. 1999. Composite mark-recapture line transect surveys. Pp. 115–126. In G. W. Garner, S. C. Amstrup, J. L. Laake, B. F. J. Manly, L. L. McDonald, and D. G. Robertson (eds.), *Marine Mammal Survey and Assessment Methods*. Balkema, Rotterdam, The Netherlands.

Borchers, D. L., W. Zucchini, and R. M. Fewster. 1998. Mark-recapture models for line transect surveys. Biometrics 54:1207–1220.

Bortolus, A. 2008. Error cascades in the biological sciences: The unwanted consequences of using bad taxonomy in ecology. Ambio 37:114–118.

Bosakowski, T. 1999. Amphibian macrohabitat associations on a private industrial forest in western Washington. Northwestern Naturalist 80:61–69.

Bostic, D. L. 1965. Home range of the Teiid lizard, *Cnemidophorus hyperythrus beldingi*. Southwestern Naturalist 10:278–281.

Bothner, R. C. 1963. A hibernaculum of the Short-headed Garter Snake, *Thamnophis brachystoma* Cope. Copeia 1963:572–573.

Boughner, J. C., M. Buchtová, K. Fu, V. Diewert, B. Hallgrímsson, and J. M. Richman. 2007. Embryonic development of *Python sebae* – I: Staging criteria and macroscopic skeletal morphogenesis of the head and limbs. Zoology 110:212–230.

Boulanger, J., and C. J. Krebs. 1994. Comparison of capture-recapture estimators of Snowshoe Hare populations. Canadian Journal of Zoology 72:1800–1807.

Bowditch, N. 1966. *American Practical Navigator.* US Naval Oceanographic Hydrographic Office, Washington, DC. Hydrographic Office Publication no. 9.

Bowerman, B. L., and R. T. O'Connell. 1993. *Forecasting and Time Series: An Applied Approach.* Rev. 3rd ed. Duxbury, Boston, Massachusetts.

Bowers, B. B., and G. M. Burghardt. 1992. The scientist and the snake: Relationships with reptiles. Pp. 250–263. In H. Davis and D. Balfour (eds.), *The Inevitable Bond: Examining Scientist-Animal Interactions.* Cambridge University Press, Cambridge, England.

Boyarski, V. L., J. A. Savidge, and G. H. Rodda. 2008. Brown Treesnake (*Boiga irregularis*) trappability: Attributes of the snake, environment and trap. Applied Herpetology 5:47–61.

Boyce, M. S. 1992. Population viability analysis. Annual Review of Ecology and Systematics 23:481–506.

Boycott, R. C., and O. Bourquin. 2000. *The Southern African Tortoise Book. A Guide to Southern African Tortoises, Terrapins, and Turtles.* O. Bourquin, KwaZulu-Natal, South Africa.

Bradshaw, C. J. A., Y. Fukuda, M. Letnic, and B. W. Brook. 2006. Incorporating known sources of uncertainty to determine precautionary harvests of saltwater crocodiles. Ecological Applications 16:1436–1448.

Braid, M. R. 1974. A bal-chatri trap for basking turtles. Copeia 1974:539–540.

Braithwaite, A. C., J. Buckley, K. F. Corbett, P. W. Edgar, E. S. Haslewood, G. A. D. Haslewood, T. E. S. Langton, and W. J. Whitaker. 1989. The distribution in England of the Smooth Snake (*Coronella austriaca laurenti*). Herpetological Journal 1:370–376.

Branch, B. 1998. *Field Guide to the Snakes and Other Reptiles of Southern Africa.* 3rd ed. Struik, Cape Town, South Africa.

Brandt, L. A. 1991. Long-term changes in a population of *Alligator mississippiensis* in South Carolina. Journal of Herpetology 25:419–424.

Braun, J., and S. P. Epperly. 1995. Aerial surveys for sea turtles in southern Georgia waters. Pp. 170–171. In J. I. Richardson and T. H. Richardson (compilers), *Proceedings of the Twelfth Annual Workshop on Sea Turtle Biology and Conservation.* NOAA Technical Memorandum NMFS-SEFC-361. National Marine Fisheries Service, Miami, Florida.

Braun, J., S. P. Epperly, and A. J. Chester. 1990. Aerial surveys for sea turtles in Core and Pamlico Sounds, North Carolina. Pp. 221–222. In T. H. Richardson, J. I. Richardson, and M. Donnelly (compilers), *Proceedings of the Tenth Annual Workshop on Sea Turtle Conservation and Biology.* NOAA Technical Memorandum NMFS-SEFC-278. National Marine Fisheries Service, Miami, Florida.

Breckenridge, W. J. 1944. *Reptiles and Amphibians of Minnesota.* University of Minnesota Press, Minneapolis, Minnesota.

Breckenridge, W. J. 1955. Observations on the life-history of the soft-shelled turtle *Trionyx ferox,* with especial reference to growth. Copeia 1955:5–9.

Brehme, C. S. 2003. Responses of Small Terrestrial Vertebrates to Roads in a Coastal Sage Scrub Ecosystem. Unpubl. Master's thesis, San Diego State University, San Diego, California.

Bricker, J., L. M. Bushar, H. K. Reinert, and L. Gelbert. 1996. Purification of high quality DNA from shed skin. Herpetological Review 27:133–134.

Brillouin, L. 1962. *Science and Information Theory.* 2nd ed. Academic Press, New York.

Brinkman, D. B., M. J. Ryan, and D. A. Eberth. 1998. The paleogeographic and stratigraphic distribution of ceratopsids (Ornithischia) in the Upper Judith River Group of western Canada. Polios 13:160–169.

Brook, B. W., L. Lim, R. Harden, and R. Frankham. 1997. Does population viability analysis software predict the behaviour of real populations? A retrospective analysis of the Lord Howe Island Woodhen *Tricholimnas sylvestris* (Sclater). Biological Conservation 82:119–128.

Brook, B. W., J. J. O'Grady, A. P. Chapman, M. A. Burgman, H. R. Akçakaya, and R. Frankham. 2000. Predictive accuracy of population viability analysis in conservation biology. Nature 404:385–387.

Brosnan, R. J., B. H. Pypendop, L. S. Barter, and M. G. Hawkins. 2006. Pharmacokinetics of inhaled anesthetics in Green Iguanas (*Iguana iguana*) American Journal of Veterinary Research 67:1670–1674.

Brown, J. H. 1995. *Macroecology.* University of Chicago Press, Chicago, Illinois.

Brown, J. H., and M. V. Lomolino. 1998. *Biogeography.* 2nd ed. Sinauer, Sunderland, Massachusetts.

Brown, L. J. 1997. An evaluation of some marking and trapping techniques currently used in the study of anuran population dynamics. Journal of Herpetology 31:410–419.

Brown, R. M., J. W. Ferner, R. V. Sison, P. C. Gonzales, and R. S. Kennedy. 1996. Amphibians and reptiles of the Zambales Mountains of Luzon Island, Republic of the Philippines. Herpetological Natural History 4:1–22.

Brown, R. M., J. A. McGuire, J. W. Ferner, N. Icarangal, Jr., and R. S. Kennedy. 2000. Amphibians and reptiles of Luzon Island, II: Preliminary report on the herpetofauna of Aurora Memorial National Park, Philippines. Hamadryad 25:175–195.

Brown, W. C., and A. C. Alcala. 1961. Populations of amphibians and reptiles in the submontane and montane forests of Cuernos de Negros, Philippine Islands. Ecology 42: 628–636.

Brown, W. C., and A. C. Alcala. 1964. Relationship of the herpetofaunas of the non-dipterocarp communities to that of the dipterocarp forest of southern Negros Island, Philippines. Senckenburgia Biologia (Frankfurt am Main) 45:591–611.

Brown, W. L., Jr. 1957. Centrifugal speciation. Quarterly Review of Biology 32:247–277.

Brown, W. S. 1991. Female reproductive ecology in a northern population of the Timber Rattlesnake, *Crotalus horridus*. Herpetologica 47:101–115.

Brown, W. S. 1993. Biology, status, and management of the Timber Rattlesnake (*Crotalus horridus*): A guide for conservation. Society for the Study of Amphibians and Reptiles, Herpetological Circular no. 22.

Brown, W. S. 2008. Sampling Timber Rattlesnakes (*Crotalus horridus*): Phenology, growth, intimidation, survival, and a syndrome of undetermined origin in a northern population. Pp. 235–256. In W. K. Hayes, K. R. Beaman, M. D. Cardwell, and S. P. Bush (eds.), *The Biology of Rattlesnakes.* Loma Linda University Press, Loma Linda, California.

Brown, W. S., V. P. J. Gannon, and D. M. Secoy. 1984. Paint-marking the rattle of rattlesnakes. Herpetological Review 15:75–76.

Brown, W. S., L. Jones, and R. Stechert. 1994. A case in herpetological conservation: Notorious poacher convicted of illegal trafficking in Timber Rattlesnakes. Bulletin of the Chicago Herpetological Society 29:74–79.

Brown, W. S., M. Kéry, and J. E. Hines. 2007. Survival of Timber Rattlesnakes (*Crotalus horridus*) estimated by capture-recapture models in relation to age, sex, color morph, time, and birthplace. Copeia 2007:656–671.

Brown, W. S., and W. S. Parker. 1976a. Movement ecology of *Coluber constrictor* near communal hibernacula. Copeia 1976:225–242.

Brown, W. S., and W. S. Parker. 1976b. A ventral scale clipping system for permanently marking snakes (Reptilia, Serpentes). Journal of Herpetology 10:247–249.

Brown, W. S., and W. S. Parker. 1982. Niche dimensions and resource partitioning in a Great Basin desert snake community. Pp. 59–81. In N. J. Scott, Jr. (ed.), *Herpetological Communities.* U.S. Department of the Interior, Fish and Wildlife Service, Wildlife Research Report 13.

Brown, W. S., W. S. Parker, and J. A. Elder. 1974. Thermal and spatial relationships of two species of colubrid snakes during hibernation. Herpetologica 30:32–38.

Bub, H. 1991. *Bird Trapping and Bird Banding: A Handbook for Trapping Methods All Over the World.* Cornell University Press, Ithaca, New York.

Buckland, S. T., D. R. Anderson, K. P. Burnham, and J. L. Laake. 1993. *Distance Sampling: Estimating Abundance of Biological Populations.* Chapman Hall, New York. http://www.colostate.edu/Dept/coopunit/download.html

Buckland, S. T., D. R. Anderson, K. P. Burnham, J. L. Laake, D. L. Borchers, and L. Thomas. 2001. *Introduction to Distance Sampling: Estimating Abundance of Biological Populations.* Oxford University Press, Oxford, United Kingdom.

Bugbee, R. E. 1945. A note on the mortality of snakes on highways in western Kansas. Transactions Kansas Academy of Science 47:373–374.

Buhlmann, K. A., and T. D. Tuberville. 1998. Use of passive integrated transponder (PIT) tags for marking small freshwater turtles. Chelonian Conservation and Biology 3:102–104.

Buhlmann, K. A., and M. R. Vaughan. 1991. Ecology of the turtle *Pseudemys concinna* in the New River, West Virginia. Journal of Herpetology 25:72–78.

Buhlmann, K., T. Tuberville, and W. Gibbons. 2008. *Turtles of the Southeast.* University of Georgia Press, Athens, Georgia.

Bull, J. J. 1980. Sex determination in reptiles. Quarterly Review of Biology 55:3–21.

Bull, J. J., and R. C. Vogt. 1979. Temperature-dependent sex determination in turtles. Science 206:1186–1188.

Burbidge, A. A. 1967. The Biology of South-Western Australian Tortoises. Unpubl. Ph.D. dissert. University of Western Australia, Perth, Western Australia, Australia.

Burgdorf, S. J., D. C. Rudolph, R. N. Conner, D. Saenz, and R. R. Schaefer. 2005. A successful trap design for capturing large terrestrial snakes. Herpetological Review 36:421–424.

Burger, J., R. T. Zappalorti, M. Gochfeld, W. I. Boarman, M. Caffrey, V. Doig, S. D. Garber, B. Lauro, M. Mikovsky, C. Safina, and J. Saliva. 1988. Hibernacula and summer den sites of Pine Snakes (*Pituophis melanoleucus*) in the New Jersey Pine Barrens. Journal of Herpetology 22:425–433.

Burghardt, G. M. 1977. Learning processes in reptiles. Pp. 555–681. In C. Gans and D. W. Tinkle (eds.), *Biology of the Reptilia*. Vol. 7. *Ecology and Behaviour A*. Academic Press, New York.

Burghardt, G. M. 2009. Ethics and animal consciousness: How rubber the ethical ruler? Journal of Social Issues 65:491–521.

Burghardt, G. M., D. Chiszar, J. B. Murphy, J. Romano, Jr., T. Walsh, and J. Manrod. 2002. Behavioral complexity, behavioral development, and play. Pp. 78–117. In J. B. Murphy, C. Ciofi, C. de la Panouse, and T. Walsh (eds.), *Komodo Dragons: Biology and Conservation*. Smithsonian Institution Press, Washington, DC.

Burghardt, G. M., and A. S. Rand (eds.). 1982. *Iguanas of the World: Their Behavior, Ecology, and Conservation*. Noyes, Park Ridge, New Jersey.

Burke, V. J, S. L. Rathbun, J. R. Bodie, and J. W. Gibbons. 1998. Effect of density on predation rate for turtle nests in a complex landscape. Oikos 83:3–11.

Burkett, D. W., and B. C. Thompson. 1994. Wildlife association with human-altered water sources in semiarid vegetation communities. Conservation Biology 8:682–690.

Burnham, K. P., and D. R. Anderson. 1984. The need for distance data in transect counts. Journal of Wildlife Management 48:1248–1254.

Burnham, K. P., and D. R. Anderson. 1998. *Model Selection and Inference: A Practical Information-Theoretic Approach*. Springer, New York.

Burnham, K. P., D. R. Anderson, and J. L. Laake. 1980. Estimation of density from line transect sampling of biological populations. Wildlife Monographs 72:3–202.

Burnham, K. P., D. R. Anderson, and J. L. Laake. 1985. Efficiency and bias in strip and line transect sampling. Journal of Wildlife Management 49:1012–1018.

Burns, G., and H. Heatwole. 1998. Home range and habitat use of the Olive Sea Snake, *Aipysurus laevis,* on the Great Barrier Reef, Australia. Journal of Herpetology 32:350–358.

Burton, E. J., A. H. Andrews, K. H. Coale, and G. M. Cailliet. 1999. Application of radiometric age determination to three long-lived fishes using ^{210}Pb:^{226}Ra disequilibria in calcified structures: A review. Pp. 77–87. In J. A. Musick (ed.), *Life in the Slow Lane: Ecology and Conservation of Long-lived Marine Animals.* American Fisheries Society Symposium 23. American Fisheries Society, Bethesda, Maryland.

Bury, R. B., and P. S. Corn. 1987. Evaluation of pitfall trapping in northwestern forests: Trap arrays with drift fences. Journal of Wildlife Management 51:112–119.

Bury, R. B., and D. J. Germano. 1998. Annual deposition of scute rings in the Western Pond Turtle, *Clemmys marmorata*. Chelonian Conservation and Biology 3:108–109.

Bury, R. B., and M. G. Raphael. 1983. Inventory methods for amphibians and reptiles. Pp. 416–419. In J. F. Bell and T. Atterbury (eds.), *Renewable Resource Inventories for Monitoring Changes and Trends: Proceedings of an International Conference, August 15–19, 1983, Corvallis, Oregon, U.S.A.* SAF 83-14. Oregon State University, College of Forestry, Corvallis, Oregon.

Buskirk, J. R. 1989. Field observations on *Phrynops williamsi* and other Uruguayan chelonians. Vivarium 1(4):8–11.

Butler, B. O., and T. E. Graham. 1993. Tracking hatchling Blanding's Turtles with fluorescent pigments. Herpetological Review 24:21–22.

Butler, J. A. 1993. Seasonal reproduction in the African Olive Grass Snake, *Psammophis phillipsi* (Serpentes: Colubridae). Journal of Herpetology 27:144–148.

Butler, P. J., P. B. Frappell, T. Wang, and M. Wikelski. 2002. The relationship between heart rate and rate of oxygen consumption in Galapagos Marine Iguanas (*Amblyrhynchus cristatus*) at two different temperatures. Journal of Experimental Biology 205:1917–1924.

Buys, A. C. 1973. Operator dangers associated with the use of immobilization drugs-accidents and emergency treatment. Pp. 77–83. In E. Young (ed.), *The Capture and Care of Wild Animals*. Human Rousseau, Cape Town, South Africa.

Buzas, M. A. 1979. The measurement of species diversity. Pp. 3–10. In J. H. Lipps, W. H. Berger, M. A. Buzas, R. G. Douglas, and C. A. Ross, *Foraminiferal Ecology and Paleoecology.* Short Course no. 6. Society of Economic Paleontologists and Mineralogists, Houston, Texas.

Cabanac, A., and M. Cabanac. 2000. Heart rate response to gentle handling of frog and lizard. Behavioural Processes 52:89–95.

Cablk, M. E., and J. S. Heaton. 2006. Accuracy and reliability of dogs in surveying for Desert Tortoise (*Gopherus agassizii*). Ecological Applications 16:1926–1935.

Cagle, F. R. 1939. A system of marking turtles for future identification. Copeia 1939:170–173.

Cagle, F. R., and A. H. Chaney. 1950. Turtle populations in Louisiana. American Midland Naturalist 43:383–388.

Cagle, N. L. 2008. Snake species distributions and temperate grasslands: A case study from the American tallgrass prairie. Biological Conservation 141:744–755.

Cairns, D. K. 1983. Examining nesting cavities with an optical fiber scope. Wilson Bulletin 95:492.

Calderwood, H. W. 1971. Anesthesia for reptiles. Journal of the American Veterinary Medical Association 159:1618–1625.

Callicott, J. B., and R. Frodeman (eds.). 2008. *Encyclopedia of Environmental Ethics and Philosophy*. Gale Group, Macmillan Reference USA, Farmington, Michigan.

Campbell, E. W., III. 1996. The Effect of Brown Tree Snake (*Boiga irregularis*) Predation on the Island of Guam's Extant Lizard Assemblages. Unpubl. Ph.D. dissert., Ohio State University, Columbus, Ohio.

Campbell, G. S. 1990. *Biophysical Measurements and Instrumentation: A Laboratory Manual for Environmental Physics*. Department of Agronomy and Soils, Washington State University, Pullman, Washington.

Campbell, H. 1953. Observations on snakes DOR in New Mexico. Herpetologica 9:157–160.

Campbell, H. 1956. Snakes found dead on the roads of New Mexico. Copeia 1956:124–125.

Campbell, H. W., and S. P. Christman. 1982. Field techniques for herpetofaunal community analysis. Pp. 193–200. In N. J. Scott, Jr. (ed.), *Herpetological Communities*. U.S. Department of the Interior, Fish and Wildlife Service, Wildlife Research Report 13.

Campbell, J. A. 1998. *Amphibians and Reptiles of Northern Guatemala, the Yucatán, and Belize*. University of Oklahoma Press, Norman, Oklahoma.

Campbell, J. A., and E. D. Brodie, Jr.(eds.). 1992. *Biology of Pitvipers.* Selva, Tyler, Texas.

Campbell, J. A., and W. W. Lamar. 2004. *The Venomous Reptiles of the Western Hemisphere*. Vols. 1–2. Comstock, Cornell University Press, Ithaca, New York.

Camper, J. D., and J. R. Dixon. 1988. *Evaluation of a microchip marking system for amphibians and reptiles.* Texas Parks and Wildlife Department. Research Publication 7100-159, 22 pp.

Campos, Z. 1993. Effect of habitat on survival of eggs and sex ratio of hatchlings of *Caiman crocodilus yacare* in the Pantanal, Brazil. Journal of Herpetology 27:127–132.

Campos, Z., M. Coutinho, and W. E. Magnusson. 2005. Field body temperatures of caimans in the Pantanal, Brazil. Herpetological Journal 15:97–106.

Campos, Z., M. Coutinho, G. Mourão, P. Bayliss, and W. E. Magnusson. 2006. Long distance movements by *Caiman crocodilus yacare*: Implications for management of the species in the Brazilian Pantanal. Herpetological Journal 16:123–132.

Campos, Z., and W. Magnusson. 1995. Relationships between rainfall, nesting habitat and fecundity of *Caiman crocodilus yacare* in the Pantanal, Brazil. Journal of Tropical Ecology 11:351–358.

Campos, Z., and G. Mourão. 1995. *Caiman latirostris* (Broad-snouted Caiman). Nesting. Herpetological Review 26:203–204.

Campos, Z., G. Mourão, M. Coutinho, and C. Abercrombie. 1994. Night-light counts, size structures, and sex ratios in wild populations of Yacare Caiman (*Caiman crocodilus yacare*) in the Brazilian Pantanal. Vida Silvestre Neotropical 4:46–50.

Cann, J. 1998. *Australian Freshwater Turtles*. Beaumont, Singapore.

Card, W., and A. G. Kluge. 1995. Hemipeneal skeleton and varanid lizard systematics. Journal of Herpetology 29:275–280.

Card, W., and D. Mehaffey. 1994. A radiographic sexing technique for *Heloderma suspectum*. Herpetological Review 25:17–19.

Cardona L., M. Revelles, C. Carreras, M. San Felix, M. Gazo, and A. Aguilar. 2005. Western Mediterranean immature Loggerhead Turtles: Habitat use in spring and summer assessed through satellite tracking and aerial surveys. Marine Biology 147:583–591.

Carlström, D., and C. Edelstam. 1946. Methods of marking reptiles for identification after recapture. Nature 158:748–749.

Carpenter A. I., J. M. Rowcliffe, and A. R. Watkinson. 2004. The dynamics of the global trade in chameleons. Biological Conservation 120:291–301.

Carpenter, C. C. 1953. A study of hibernacula and hibernating associations of snakes and amphibians in Michigan. Ecology 34:74–80.

Carpenter, C. C. 1955. Sounding turtles: A field locating technique. Herpetologica 11:120.

Carpenter, C. C. 1957. Hibernation, hibernacula and associated behavior of the Three-toed Box Turtle (*Terrapene carolina triunguis*). Copeia 1957:278–282.

Carpenter, C. C. 1982. The Bullsnake as an excavator. Journal of Herpetology 16:394–401.

Carr, A. 1952. *Handbook of Turtles. The Turtles of the United States, Canada, and Baja California*. Comstock, Cornell University Press, Ithaca, New York.

Carr, A. F., Jr., and L. J. Marchand.1942. A new turtle from the Chipola River, Florida. *Proceedings of the New England Zoölogical Club* 20:95–100.

Carr, D., and P. H. Carr. 1977. Survey and Reconnaissance of Nesting Shores and Coastal Habitats of Marine Turtles in Florida, Puerto Rico, and the US Virgin Islands. Unpubl. Contract report to US National Marine Fisheries Service, Contract no. 0360423519, St. Petersburg, Florida.

Carreira, S., M. Meneghel, and F. Achaval. 2005. *Reptiles de Uruguay*. Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

Carroll, S. B., J. K. Grenier, and S. D. Weatherbee. 2001. *From DNA to Diversity: Molecular Genetics and the Evolution of Animal Design*. Blackwell, Malden, Massachusetts.

Carson, D. 2000. Relative abundance and distribution of sea turtles in the marine and estuarine waters of Palm Beach County, Florida, USA based on aerial surveys, 1990–1993. Pp. 148–152. In H. J. Kalb and T. Wibbels (compilers), *Proceedings of the Nineteenth Annual Symposium on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFSC-443. National Marine Fisheries Service, Miami, Florida.

Caruso, T., and M. Migliorini. 2006. A new formulation of the geometric series with applications to oribatid (Acari, Oribatida) species assemblages from human-disturbed Mediterranean areas. Ecological Modeling 195:402–406.

Casazza, M. L., G. D. Wylie, and C. J. Gregory. 2000. A funnel trap modification for surface collection of aquatic amphibians and reptiles. Herpetological Review 31:91–92.

Case, T. J., and R. N. Fisher. 2001. Measuring and predicting species presence: Coastal sage scrub case study. Pp. 47–71. In C. T. Hunsaker, M. F. Goodchild, M. A. Friedl, and T. J. Case (eds.), *Spatial Uncertainty in Ecology: Implications for Remote Sensing and GIS Applications*. Springer, New York.

Cassey, P., and G. T. Ussher. 1999. Estimating abundance of tuatara. Biological Conservation 88:361–366.

Castanet, J., and M. Cheylan. 1979. Les marques de croissance des os et des écailles comme indicateur de l'âge chez *Testudo hermanni* et *Testudo graeca* (Reptilia, Chelonia, Testudinidae). Canadian Journal of Zoology 57:1649–1665.

Castanet, J., H. Francillon-Vieillot, F. J. Meunier, and A. de Ricqlès. 1993. Bone and individual aging. Pp. 245–283. In B. K. Hall (ed.), *Bone*. Vol. 7: *Bone Growth-B*. CRC Press, Boca Raton, Florida.

Castanet, J., and G. Naulleau. 1985. La squelettochronologie chez les reptiles. II. Résultats expérimentaux sur la signification des marques de croissance squelettiques chez les serpents. Remarques sur la croissance et la longévité de la Vipère Aspic. Annales des Sciences Naturelles, Zoologie et Biologie animale, Paris. Series 13, 7:41–62.

Castanet, J., D. G. Newman, and H. Saint Girons. 1988. Skeletochronological data on the growth, age, and population structure of the Tuatara, *Sphenodon punctatus,* on Stephens and Lady Alice Islands, New Zealand. Herpetologica 44:25–37.

Castilla, A. M., and D. Bauwens. 2000. Reproductive characteristics of the lacertid lizard *Podarcis atrata*. Copeia 2000:748–756.

Caswell, H. 2001. *Matrix Population Models: Construction, Analysis, and Interpretation*. 2nd ed. Sinauer, Sunderland, Massachusetts.

Caughley, G., and A. R. E. Sinclair. 1994. *Wildlife Ecology and Management*. Blackwell, Cambridge, Massachusetts.

Ceballos Fonseca, C. P. 2001. The Native and Exotic Freshwater Turtle and Tortoise Trade in Texas. Unpubl. Master's thesis, Texas A & M University, College Station, Texas.

Ceballos, C. P., and L. A. Fitzgerald. 2004. The trade in native and exotic turtles in Texas. Wildlife Society Bulletin 32:881–892.

Cei, J. M. 1993. *Reptiles del Noroeste, Nordeste y Este de la Argentina. Herpetofauna de las selvas subtropicales, Puna y Pampas*. Monografie XIV, Museo Regionale di Scienze Naturali, Torino, Italy.

CETAP (Cetacean and Turtle Assessment Program, University of Rhode Island). 1982. *A Characterization of Marine Mammals and Turtles in the Mid- and North-Atlantic Areas of the US Outer Continental Shelf, Final Report*. Contract no. AA551-CT8-48. USDI Bureau of Land Management, Washington, DC.

Chabreck, H. 1966. Methods of determining population size and composition of alligator populations in Louisiana. Pp. 105–112. In *Proceedings of the Twentieth Annual Conference, Southeastern Association of Game and Fish Commissioners, 1966*.

Chabreck, R. H. 1965. Methods of capturing, marking and sexing alligators. Pp. 47–50. In *Proceedings of the Seventeenth Annual Conference, Southeastern Association of Game and Fish Commissioners, 1963*.

Chan-ard, T., W. Grossmann, A. Gumprecht, and K.-D. Schulz. 1999. *Amphibians and Reptiles of Peninsular Malaysia and Thailand*. Bushmaster, Wuerselen, Germany.

Chaney, A., and C. L. Smith. 1950. Methods for collecting Mapturtles. Copeia 1950:323–324.

Chapple, D. G. 2003. Ecology, life-history, and behavior in the Australian scincid genus *Egernia,* with comments on the evolution of complex sociality in lizards. Herpetological Monographs 17:145–180.

Charland, M. B. 1991. Anesthesia and transmitter implantation effects on gravid garter snakes (*Thamnophis sirtalis* and *T. elegans*). Herpetological Review 22:46–47.

Cherkiss, M. S., H. E. Fling, F. J. Mazzotti, and K. G. Rice. 2004. *Counting and Catching Crocodilians*. Department of Wildlife Ecology and Conservation, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida, Circular 1451. http://edis.ifas.ufl.edu/uw198

Cherkiss, M. S., F. J. Mazzotti, and K. G. Rice. 2006. Effects of shoreline vegetation on visibility of American Crocodiles (*Crocodylus acutus*) during spotlight surveys. Herpetological Review 37:37–40.

Chiaraviglio, M., M. Sironi, R. Cervantes, M. Bertona, and S. Lucino. 1998. Ultrasound imaging of the reproductive structures in *Boa constrictor occidentalis* (Serpentes: Boidae). Gayana Zoología 62:91–95.

Chinsamy, A., S. A. Hanrahan, R. M. Neto, and M. Seely. 1995. Skeletochronological assessment of age in *Angolosaurus skoogi,* a cordylid lizard living in an aseasonal environment. Journal of Herpetology 29:457–460.

Chippaux, J.-P. 1999. *Les Serpents d'Afrique Occidental et Centrale.* l'IRD, Paris, France.

Chrisman, N. R. 1987. The accuracy of map overlays: A reassessment. Landscape and Urban Planning 14:427–439.

Christiansen, J. L., and T. Vandewalle. 2000. Effectiveness of three trap types in drift fence surveys. Herpetological Review 31:158–160.

Christy, B. 2010. The Serpent King. Foreign Policy, December 28, 2010. http://www.foreignpolicy.com/articles/2010/12/28/the_serpent_king

Ciampaglio, C. N., M. Kemp, and D. W. McShea. 2001. Detecting changes in morphospace occupation patterns in the fossil record: Characterization and analysis of measures of disparity. Paleobiology 27:695–715.

Ciofi, C., and G. Chelazzi. 1991. Radiotracking of *Coluber viridiflavus* using external transmitters. Journal of Herpetology 25:37–40.

CITES Secretariat. 2010. *CITES: Convention on International Trade in Endangered Species of Wild Fauna and Flora.* CITES Secretariat, United Nations Environment Programme, Geneva, Switzerland. http://www.cites.org/ [accessed November 2010].

Clark, A. 1998. Reptile sheds yield high quality DNA. Herpetological Review 29:17–18.

Clark, D. L., and J. C. Gillingham. 1984. A method for nocturnally locating lizards. Herpetological Review 15:24–25.

Clark, T. W., and T. M. Campbell III. 1983. A small carnivore survey technique. Great Basin Naturalist 43:438–440.

Clarke, J. A., J. T. Chopko, and S. P. Mackessy. 1996. The effect of moonlight on activity patterns of adult and juvenile Prairie Rattlesnakes (*Crotalus viridis viridis*). Journal of Herpetology 30:192–197.

Clarke, K. C. 1997. *Getting Started with Geographic Information Systems.* 2nd ed. Prentice Hall, Upper Saddle River, New Jersey.

Claussen, D. L., M. S. Finkler, and M. M. Smith. 1997. Thread trailing of turtles: Methods for evaluating spatial movements and pathway structure. Canadian Journal of Zoology 75:2120–2128.

Clifford, H. T., and W. Stephenson. 1975. *An Introduction to Numerical Classification.* Academic Press, New York.

Cobb, V. A. 1994. The Ecology of Pregnancy in Free-Ranging Great Basin Rattlesnakes (*Crotalus viridis lutosus*). Unpubl. Ph.D. dissert., Idaho State University, Pocatello, Idaho.

Coddington, E. J., and A. Cree. 1997. Population numbers, response to weather, movements and management of the threatened New Zealand skinks *Oligosoma grande* and *O. otagense* in tussock grassland. Pacific Conservation Biology 3:379–391.

Cody, M. L. 1996. Introduction to long-term community ecological studies. Pp. 1–15. In M. L. Cody and J. A. Smallwood (eds.), *Long-Term Studies of Vertebrate Communities.* Academic Press, New York.

Cogger, H. G. 1984. Reptiles in the Australian arid zone. Pp. 235–252. In H. G. Cogger and E. E. Cameron (eds.), *Arid Australia.* Australian Museum, Sydney, Australia.

Cogger, H., H. Heatwole, Y. Ishikawa, M. McCoy, N. Tamiya, and T. Teruuchi. 1987. The status and natural history of the Rennell Island Sea Krait, *Laticauda crockeri* (Serpentes: Laticaudidae). Journal of Herpetology 21:255–266.

Cohen, E. 1948. Emergence of *Coluber c. constrictor* from hibernation. Copeia 1948:137–138.

Cohen, J. 1977. *Statistical Power Analysis for the Behavioral Sciences.* Academic Press, New York.

Coleman, J. L., N. B. Ford, and K. Herriman. 2008. A road survey of amphibians and reptiles in a bottomland hardwood forest. Southeastern Naturalist 7:339–348.

Coles, W. C., J. A. Keinath, D. E. Barnard, and J. A. Musick. 1994. Sea surface temperatures and sea turtle position correlations. Pp. 211–212. In K. A. Bjorndal, A. B. Bolten, D. A. Johnson, and P. J. Eliazar (compilers). *Proceedings of the Fourteenth Annual Symposium on Sea Turtle Biology and Conservation.* NOAA Technical Memorandum NMFS-SEFSC-351. National Marine Fisheries Service, Miami, Florida.

Collins, E. P., and G. H. Rodda. 1994. Bone layers associated with ecdysis in laboratory-reared *Boiga irregularis* (Colubridae). Journal of Herpetology 28:378–381.

Colwell, R. K. 2007. *Biota 2: The Biodiversity Database Manager.* Sinauer, Sunderland, Massachusetts. http://viceroy.eeb.uconn.edu/biota

Colwell, R. K. 2009. *Estimates: Statistical Estimation of Species Richness and Shared Species from Samples.* Vers. 8.2. http://viceroy.eeb.uconn.edu/estimates

Colwell, R. K., and J. A. Coddington. 1995. Estimating terrestrial biodiversity through extrapolation. Pp. 101–118. In D. L. Hawksworth (ed.), *Biodiversity: Measurement and Estimation.* Chapman Hall, New York.

Conant, R. 1969. A review of the water snakes of the genus *Natrix* in Mexico. Bulletin of the American Museum of Natural History 142:1–140.

Conant, R., and J. T. Collins. 1998. *A Field Guide to Reptiles: Eastern and Central North America.* 3rd ed. Houghton Mifflin, Boston, Massachusetts.

Conant, R., M. B. Trautman, and E. B. McLean. 1964. The False Map Turtle, *Graptemys pseudogeographica* (Gray), in Ohio. Copeia 1964:212–213.

Congdon, J. D., R. E. Ballinger, and K. A. Nagy. 1979. Energetics, temperature and water relations in winter aggregated *Sceloporus jarrovi* (Sauria: Iguanidae). Ecology 60:30–35.

Congdon, J. D., A. E. Dunham, and R. C. van Loben Sels. 1994. Demographics of Common Snapping Turtles (*Chelydra serpentina*): Implications for conservation and management of long-lived organisms. American Zoologist 34:397–408.

Congdon, J. D., R. U. Fischer, and R. E. Gatten, Jr. 1995. Effects of incubation temperatures on characteristics of hatchling American Alligators. Herpetologica 51:497–504.

Congdon, J. D., and J. W. Gibbons. 1996. Structure and dynamics of a turtle community over two decades. Pp. 137–159. In M. L. Cody and J. A. Smallwood (eds.), *Long-Term Studies of Vertebrate Communities.* Academic Press, New York.

Conner, M. C., R. F. Labisky, and D. R. Progulske, Jr. 1983. Scent-station indices as measures of population abundance for bobcats, raccoons, gray foxes, and opossums. Wildlife Society Bulletin 11:146–152.

Cooch, E. G., and G. C. White. 2005. *Program MARK: A Gentle Introduction.* [online; now = 2010, 9th ed.] http://www.phidot.org/software/mark/docs/book/

Cooper, J. E. 1974. Ketamine hydrochloride as an anaesthetic for East African reptiles. Veterinary Record 95:37–41.

Cooper, M. E. 2008. Forensics in herpetology—legal aspects. Applied Herpetology 5:319–338.

Cooper, W. E., Jr., and N. Greenberg. 1992. Reptilian coloration and behavior. Pp. 298–422. In C. Gans and D. Crews (eds.), *Biology of the Reptilia*. Vol. 18. *Physiology E, Hormones, Brain and Behavior.* University of Chicago Press, Chicago, Illinois.

Cooper, W. E., Jr., and L. J. Vitt. 1988. Orange head coloration of the male Broad-headed Skink (*Eumeces laticeps*), a sexually selected social cue. Copeia 1988:1–6.

Cooper-Preston, H. 1992. Geographic Variation in the Population Dynamics of *Crocodylus johnstoni* (Krefft) in Three Rivers in the Northern Territory, Australia. Unpubl. Ph.D. dissert., University of New England, Armidale, New South Wales, Australia.

Cooperrider, A. Y., R. J. Boyd, and H. R. Stuart (eds.). 1986. Inventory and monitoring of wildlife habitat. Bureau of Land Management, U.S. Department of Interior, Washington, DC. http://www.ntc.blm.gov/krc/viewresource.php?courseID=255

Corben, C., and G. M. Fellers. 2001. A technique for detecting eyeshine of amphibians and reptiles. Herpetological Review 32:89–91.

Corn, P. S. 1994. 7. Straight-line drift fences and pitfall traps. Pp. 109–117. In W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster (eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians.* Smithsonian Institution Press, Washington, DC.

Corn, P. S., and R. B. Bury. 1990. *Sampling Methods for Terrestrial Amphibians and Reptiles.* U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, General Technical Report PNW-GTR-256.

Cott, H. B. 1961. Scientific results of an inquiry into the ecology and economic status of the Nile Crocodile (*Crocodilus niloticus*) in Uganda and Northern Rhodesia. Transactions of the Zoological Society of London 29:211–357.

Cotterill, F. P. D. 1995. Systematics, biological knowledge and environmental conservation. Biodiversity and Conservation 4:183–205.

Cowardin, L. M., V. Carter, F. C. Golet, and E. T. LaRoe. 1979. Classification of wetlands and deepwater habitats of the United States. US Fish and Wildlife Service, Biological Services Program, FWS/OBS-79/31.

Cowles, R. B. 1941. Observations on the winter activities of desert reptiles. Ecology 22:125–140.

Cowles, R. B., and C. M. Bogert. 1944. A preliminary study of the thermal requirements of desert reptiles. Bulletin of the American Museum of Natural History 83:261–296.

Cox, M. J., P. P. Van Dijk, J. Nabhitabhata, and K. Thirakhupt. 2010. *A Photographic Guide to Snakes and Other Reptiles of Peninsular Malaysia, Singapore and Thailand.* New Holland, London, United Kingdom.

Crastz, F. 1982. Embryological stages of the marine turtle *Lepidochelys olivacea* (Eschscholtz). Revista de Biología Tropical 30:113–120.

Crawford, E., and A. Kurta. 2000. Color of pitfall affects trapping success for anurans and shrews. Herpetological Review 31:222–224.

Crawford, K. M., J. R. Spotila, and E. A. Standora. 1983. Operative environmental temperatures and basking behavior of the turtle *Pseudemys scripta*. Ecology 64:989–999.

Cree, A., J. F. Cockrem, M. A. Brown, P. R. Watson, L. J. Guillette Jr., D. G. Newman, and G. K. Chambers. 1991. Laparoscopy, radiography, and blood analyses as techniques for identifying the reproductive condition of female tuatara. Herpetologica 47:238–249.

Cressie, N. A. C. 1993. *Statistics for Spatial Data*. Rev. ed. Wiley, New York.

Criddle, S. 1937. Snakes from an ant hill. Copeia 1937:142.

Croak, B. M., D. A. Pike, J. K. Webb, and R. Shine. 2010. Using artificial rocks to restore nonrenewable shelter sites in human-degraded systems: Colonization by fauna. Restoration Ecology 18:428–438. [Published Online: Dec 4 2008. doi: 10.1111/j.1526-100X.2008.00476.x].

Cross, C. L. 1998. Ecology of the Eastern Cottonmouth (*Agkistrodon p. piscivorus*) at Back Bay National Wildlife Refuge: A Comparative Study of Natural and Anthropogenic Marsh Habitats. Unpubl. Ph.D. dissert. Old Dominion University, Norfolk, Virginia.

Cross, C. L. 2000. A new design for a lightweight squeeze box for snake field studies. Herpetological Review 31:34.

Crother, B. I. (ed.). 1999. *Caribbean Amphibians and Reptiles*. Academic Press, San Diego, California.

Crother, B. I. (ed.). 2008 (as updated 31 March 2011). *Scientific and Standard English Names of Amphibians and Reptiles of North America North of Mexico*. Society for the Study of Amphibians and Reptiles, Herpetological Circular no. 37. http://www.ssarherps.org/pages/comm_names/Index.php

Crouse, D. T. 1984. Loggerhead Sea Turtle nesting in North Carolina: Applications of an aerial survey. Biological Conservation 29:143–155.

Crouse, D. T. 1999. Population modeling and implications for Caribbean Hawksbill Sea Turtle management. Chelonian Conservation and Biology 3:185–188.

Crouse, D. T., L. B. Crowder, and H. Caswell. 1987. A stage-based population model for Loggerhead Sea Turtles and implications for conservation. Ecology 68:1412–1423.

Crowder, L. B., D. T. Crouse, S. S. Heppell, and T. H. Martin. 1994. Predicting the impact of turtle excluder devices on Loggerhead Sea Turtle populations. Ecological Applications 4:437–445.

Crowder, L. B., S. R. Hopkins-Murphy, and J. A. Royle. 1995. Effects of turtle excluder devices (TEDs) on Loggerhead Sea Turtle strandings with implications for conservation. Copeia 1995:773–779.

Crump, M. L., and N. J. Scott, Jr. 1994. 2. Visual encounter surveys. Pp. 84–92. In W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster (eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Smithsonian Institution Press, Washington, DC.

Cryer J. D., and K.-S. Chan. 2008. *Time series analysis: With applications in R*. 2nd ed. Springer, New York.

Cuadrado, M., I. Molina-Prescott, and L. Flores. 2003. Comparison between tail and jugular venipuncture techniques for blood sample collection in common chameleons (*Chamaeleo chamaeleon*). Veterinary Journal 166:93–97.

Culotta, W. A., and G. V. Pickwell. 1993. *The Venomous Sea Snakes: A Comprehensive Bibliography*. Krieger, Malabar, Florida.

Cunha, O. R., and F. P. Nascimento. 1978. Ofídios da Amazônia X - As cobras da região leste do Pará. Publicações Avulsas do Museu Paraense Emílio Goeldi 31:1–218.

Custer, R. S., and M. Bush. 1980. Physiologic and acid-base measures of Gopher Snakes during ketamine or halothane-nitrous oxide anesthesia. Journal of the American Veterinary Medical Association 177:870–874.

Dallwitz. M. J. 1980. *DELTA - DEscription Language for Taxonomy*. Adelaide, South Australia, Australia. http://delta-intkey.com

Dalrymple G. H., T. M. Steiner, R. J. Nodell, and F. S. Bernardino, Jr. 1991. Seasonal activity of the snakes of Long Pine Key, Everglades National Park. Copeia 1991:294–302.

Darevsky, I. S., L. A. Kupriyanova, and T. Uzzell. 1985. Parthenogenesis in reptiles. Pp. 411–526. In C. Gans and F. Billett (eds.), *Biology of the Reptilia* Vol. 15. *Development B*. John Wiley, New York.

Das, I. 2004. *A Pocket Guide: Lizards of Borneo*. Natural History Publications (Borneo), Sdn Bhd., Kota Kinabalu, Borneo, Malaysia.

Das, I. 2010. *A Field Guide to the Reptiles of South-east Asia*. New Holland, London, United Kingdom.

Das, I., and V. Wallach. 1998. Scolecophidian arboreality revisited. Herpetological Review 29:15–16.

Da Silveira, R., and W. E. Magnusson. 1999. Diets of Spectacled and Black Caiman in the Anavilhanas Archipelago, central Amazonia, Brazil. Journal of Herpetology 33:181–192.

Da Silveira, R., W. E. Magnusson, and J. B. Thorbjarnarson. 2008. Factors affecting the number of caimans seen during spotlight surveys in the Mamirauá Reserve, Brazilian Amazonia. Copeia 2008:425–430.

David, P., and I. Ineich. 1999. Les serpents venimeux du monde: Systématique et répartition. Dumerilia 3:3–499.

David, P., and G. Vogel. 1996. *The Snakes of Sumatra: An Annotated Checklist and Key with Natural History Notes*. Chimaira, Frankfurt am Main, Germany.

David, P., and G. Vogel. 2010. *Venomous Snakes of Europe, Northern, Central, and Western Asia*. Chimaira, Frankfurt am Main, Germany.

Davis, A. R., and D. H. Leavitt. 2007. Candlelight *vigilis*: A noninvasive method for sexing small, sexually monomorphic lizards. Herpetological Review 38:402–404.

Davis, G. E., and M. C. Whiting. 1977. Loggerhead sea turtle nesting in Everglades National Park, Florida, USA. Herpetologica 33:18–28.

Davis, R. B., and L. G. Phillips, Jr. 1991. A method of sexing the Dumeril's Monitor, *Varanus dumerili*. Herpetological Review 22:18–19.

Day, T. A., and R. G. Wright. 1985. The vegetation types of Craters of the Moon National Monument. University of Idaho, College of Forestry, Wildlife, and Range Sciences, Bulletin no. 38.

Deavers, D. R. 1972. Water and electrolyte metabolism in the arenicolous lizard *Uma notata notata*. Copeia 1972:109–122.

DeBenedictus, P. A. 1973. On the correlations between certain diversity indices. American Naturalist 107:295–302.

de Buffrénil, V. 1982. Morphogenesis of bone ornamentation in extant and extinct crocodilians. Zoomorphology 99:155–166.

de Caprariis, P., R. H. Lindemann, and C. M. Collins. 1976. A method for determining optimum sample size in species diversity studies. Journal of the International Association for Mathematical Geology 8:575–581.

DeGraaf, R. M., and D. D. Rudis. 1990. Herpetofaunal species composition and relative abundance among three New England forest types. Forest Ecology and Management 32:155–165.

DeGregorio, B. A., E. J. Nordberg, K. E. Stepanoff, and J. E. Hill. 2010. Patterns of snake road mortality on an isolated barrier island. Herpetological Conservation and Biology 5:441–448.

de Lang, R., and G. Vogel. 2005. *The Snakes of Sulawesi: A Field Guide to the Land Snakes of Sulawesi with Identification Keys*. Chimaira, Frankfurt am Main, Germany.

DeLaune, M. G. 2000. *XTools ArcView Extension* (Version 10/18/2000). Oregon Department of Forestry, Salem, Oregon. http://www.odf.state.or.us/divisions/management/state_forests/XTools.asp

Delgado Garcia, J. D., J. R. Arévalo, and J. M. Fernández-Palacios. 2007. Road edge effect on abundance of the lizard *Gallotia galloti* (Sauria: Lacertidae) in two Canary Islands forests. Biodiversity and Conservation 16:2949–2963.

Dellinger, T., and G. von Hegel. 1990. Sex identification through cloacal probing in juvenile marine iguanas (*Amblyrhynchus cristatus*). Journal of Herpetology 24:424–426.

Demas, S., M. Duronslet, S. Wachtel, C. Caillouet, and D. Nakamura. 1990. Sex-specific DNA in reptiles with temperature sex determination. Journal of Experimental Zoology 253:319–324.

deMaynadier, P. G., and M. L. Hunter, Jr. 2000. Road effects on amphibian movements in a forested landscape. Natural Areas Journal 20:56–65.

DeMers, M. N. 2000. *Fundamentals of Geographic Information Systems*. 2nd ed. Wiley, New York.

DeNardo, D. 1996. Reproductive biology. Pp. 212–224. In D. R. Mader (ed.), *Reptile Medicine and Surgery*. Saunders, Philadelphia, Pennsylvania.

DeNardo, D. F., and K. Autumn. 2001. Effect of male presence on reproductive activity in captive female Blood Pythons, *Python curtus*. Copeia 2001:1138–1141.

Dendy, A. 1899. Outlines of the development of the tuatara, *Sphenodon (Hatteria) punctatus*. Quarterly Journal of Microscopical Science, ser. 2, 42:1–87.

Dennis B., P. L. Munholland, and J. M. Scott. 1991. Estimation of growth and extinction parameters for endangered species. Ecological Monographs 61:115–143.

Deraniyagala, P. E. P. 1939. *The Tetrapod Reptiles of Ceylon*. Vol. 1. *Testudinates and Crocodilians*. Dulau, London, England.

Dessauer, H. C., C. J. Cole, and M. S. Hafner. 1996. Collection and storage of tissues. Pp. 29–47. In D. M. Hillis, C. Moritz, and B. K. Mable (eds.), *Molecular Systematics*. 2nd ed. Sinauer, Sunderland, Massachusetts.

Dessauer, H. C., and M. S. Hafner (eds.). 1984. *Collections of Frozen Tissues: Value, Management, Field, and Laboratory Procedures, and Directory of Existing Collections*. Association of Systematics Collections, Lawrence, Kansas.

DeVault, T. L., and O. E. Rhodes, Jr. 2002. Identification of vertebrate scavengers of small mammal carcasses in a forested landscape. Acta Theriologica 47:185–192.

Devine, M. C. 1975. Copulatory plugs in snakes: Enforced chastity. Science 187:844–845.

Devine, M. C. 1977. Copulatory plugs, restricted mating opportunities and reproductive competition among male garter snakes. Nature 267:345–346.

DeVries, D. R., and R. V. Frie. 1996. Determination of age and growth. Pp. 483–512. In B. R. Murphy and D. W. Willis (eds.), *Fisheries Techniques*. 2nd ed. American Fisheries Society, Bethesda, Maryland.

Dhouailly, D., and R. Saxod. 1974. Les stades du développement de *Lacerta muralis* Laur. entre la ponte et l'éclosion. Bulletin de la Société Zoologique de France 99:489–494.

Dickinson, H. C., J. E. Fa, and S. M. Lenton. 2001. Microhabitat use by a translocated population of St. Lucia Whiptail Lizards (*Cnemidophorus vanzoi*). Animal Conservation 4:143–156.

Diemer, J. E. 1992. Demography of the tortoise *Gopherus polyphemus* in northern Florida. Journal of Herpetology 26:281–289.

Diggle, P. J. 1990. *Time Series: A Biostatistical Introduction*. Clarendon, Oxford University Press, Oxford, England.

Diller, L. V., and R. L. Wallace. 1999. Distribution and habitat of *Ascaphus truei* in streams on managed, young growth forests in north coastal California. Journal of Herpetology 33:71–79.

Diller, L. V., and R. L. Wallace 2002. Growth, reproduction, and survival in a population of *Crotalus viridis oreganus* in north central Idaho. Herpetological Monographs 16:26–45.

Disi, A. M., D. Modrý, P. Nečas, and L. Rifai. 2001. *Amphibians and Reptiles of the Hashemite Kingdom of Jordan*. Chimaira, Frankfurt am Main, Germany.

Divers, S. J. 1996. The use of propofol in reptile anesthesia. *Proceedings of the Association of Amphibian and Reptilian Veterinarians* 1996:57–59.

Dixon, J. R. 2000. *Amphibians and Reptiles of Texas*. 2nd ed. Texas A & M University Press, College Station, Texas.

Dixon, J. R., and A. A. Yanosky. 1993. A microchip marking system for identification of caiman hatchlings. Bulletin of the Maryland Herpetological Society 29:156–159.

Doan, T. M. 2003. Which methods are most effective for surveying rain forest herpetofauna? Journal of Herpetology 37:72–81.

Doan, T. M., and W. Arizábal Arriaga. 2002. Microgeographic variation in species composition of the herpetofaunal communities of Tambopata Region, Peru. Biotropica 34:101–117.

Dobie, J. L. 1971. Reproduction and growth in the Alligator Snapping Turtle, *Macroclemys temmincki* (Troost). Copeia 1971:645–658.

Dobiey, M., and G. Vogel. 2007. *Venomous Snakes of Africa*. Chimaira, Frankfurt am Main, Germany.

Dodd, C. K., Jr. 1991. Drift fence-associated sampling bias of amphibians at a Florida sandhills temporary pond. Journal of Herpetology 25:296–301.

Dodd, C. K., Jr. 1992a. Biological diversity of a temporary pond herpetofauna in north Florida sandhills. Biodiversity and Conservation 1:125–142.

Dodd, C. K., Jr. 1992b. Fluorescent powder is only partially successful in tracking movements of the Six-Lined Racerunner (*Cnemidophorus sexlineatus*). Florida Field Naturalist 20:8–14.

Dodd, C. K., Jr. 1993a. Population structure, body mass, activity, and orientation of an aquatic snake (*Seminatrix pygaea*) during a drought. Canadian Journal of Zoology 71:1281–1288.

Dodd, C. K., Jr. 1993b. The effects of toeclipping on sprint performance of the lizard *Cnemidophorus sexlineatus*. Journal of Herpetology 27:209–213.

Dodd, C. K., Jr. 1995. Disarticulation of turtle shells in north-central Florida: How long does a shell remain in the woods? American Midland Naturalist 134:378–387.

Dodd, C. K., Jr., W. J. Barichivich, and L. L. Smith. 2004. Effectiveness of a barrier wall and culverts in reducing wildlife mortality on a heavily traveled highway in Florida. Biological Conservation 118:619–631.

Dodd, C. K., Jr., K. M. Enge, and J. N. Stuart. 1989. Reptiles on highways in north-central Alabama, USA. Journal of Herpetology 23:197–200.

Dodd, C. K., Jr., and D. E. Scott. 1994. 9. Drift fences encircling breeding sites. Pp. 125–130. In W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster (eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Smithsonian Institution Press, Washington, DC.

Dodd, F., and R. R. Capranica. 1992. A comparison of anesthetic agents and their effects on the response properties of the peripheral auditory system. Hearing Research 62:173–180.

Dodd, M. G., and A. H. Mackinnon. 2002. Spatial and temporal distribution of leatherback turtles observed off the coast of Georgia (USA), 1999. Pp. 269–271. In A. Mosier, A. Foley, and B. Brost (eds.), *Proceedings of the Twentieth Annual Symposium on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFSC-477. National Marine Fisheries Service, Miami, Florida.

Donnelly, M. A., M. H. Chen, and G. G. Watkins. 2004. Sampling amphibians and reptiles in the Iwokrama Forest ecosystem. *Proceedings of the Academy of Natural Sciences of Philadelphia* 154:55–69.

Donnelly, M. A., and C. Guyer. 1994. Mark-recapture. Pp. 183–200. In W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster (eds.). *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Smithsonian Institution Press, Washington, DC.

Doody, J. S., and J. W. Tamplin. 1992. An efficient marking technique for softshelled turtles. Herpetological Review 23:54–56.

Doody, J. S., P. West, and A. Georges. 2003. Beach selection in nesting Pig-Nosed Turtles, *Carettochelys insculpta*. Journal of Herpetology 37:178–182.

Dorazio, R. M., and J. A. Royle. 2003. Mixture models for estimating the size of a closed population when capture rates vary among individuals. Biometrics 59:351–364.

Dorazio, R. M., and J. A. Royle. 2005. Rejoinder to "The performance of mixture models in heterogeneous closed population capture-recapture." Biometrics 61:874–876.

Dorcas, M. E., and C. R. Peterson. 1998. Daily body temperature variation in free-ranging Rubber Boas. Herpetologica 54:88–103.

Dorcas, M. E., J. D. Willson, and J. W. Gibbons. 2007. Crab trapping causes population decline and demographic changes in Diamondback Terrapins over two decades. Biological Conservation 137:334–340.

Doughty, P., and R. Shine. 1997. Detecting life history trade-offs: Measuring energy stores in "capital" breeders reveals costs of reproduction. Oecologia 110:508–513.

Douglas, M. E. 1979. Migration and sexual selection in *Ambystoma jeffersonianum*. Canadian Journal of Zoology 57:2303–2310.

Dowling, H. G., and J. M. Savage. 1960. A guide to the snake hemipenis: A survey of basic structure and systematic characteristics. Zoologica, Scientific Contributions of the New York Zoological Society 45:17–28 + III plates.

Downes, S., and D. Bauwens. 2002. An experimental demonstration of direct behavioural interference in two Mediterranean lacertid lizard species. Animal Behaviour 63:1037–1046.

Downes, S., and P. Borges. 1998. Sticky traps: An effective way to capture small terrestrial lizards. Herpetological Review 29:94–95.

Downes, S., and R. Shine. 1998a. Sedentary snakes and gullible geckos: Predator-prey coevolution in nocturnal rock-dwelling reptiles. Animal Behaviour 55:1373–1385.

Downes, S., and R. Shine. 1998b. Heat, safety or solitude? Using habitat selection experiments to identify a lizard's priorities. Animal Behaviour 55:1387–1396.

Drda, W. J. 1968. A study of snakes wintering in a small cave. Journal of Herpetology 1:64–70.

Drost, C. A., T. B. Persons, and E. M. Nowak. 2001. Herpetofauna survey of Petrified Forest National Park, Arizona. Pp. 83–102. In C. van Riper, III, K. A. Thomas, and M. A. Stuart (eds.), *Proceedings of the Fifth Biennial Conference of Research on the Colorado Plateau*. U.S. Geological Survey/FRESC [Forest and Rangeland Ecosystem Science Center] Report Series USGSFRESC/COPL/2001/24.

Dubois, A., and A. Nemésio. 2007. Does nomenclatural availability of nomina of new species or subspecies require the deposition of vouchers in collections? Zootaxa 1409:1–22.

Duellman, W. E. 1999. Perils of permits: Procedures and pitfalls. Herpetological Review 30:12–16.

Duellman, W. E. 2005. *Cusco Amazónico: The Lives of Amphibians and Reptiles in an Amazonian Rainforest*. Comstock, Cornell University Press, Ithaca, New York.

Dufaure, J. P., and J. Hubert. 1961. Table de développement du lézard vivipare: *Lacerta (Zootoca) vivipara* Jacquin. Archives d'Anatomie Microscopique et de Morphologie Expérimentale 50:309–328.

Duncan, R. P., T. M. Blackburn, and T. H. Worthy. 2002. Prehistoric bird extinctions and human hunting. *Proceedings of the Royal Society of London, B. Biological Sciences* 269: 517–521.

Dundee, H. A., and D. A. Rossman. 1989. *The Amphibians and Reptiles of Louisiana*. Louisiana State University Press, Baton Rouge, Louisiana.

Dunham, A. E., and J. W. Gibbons. 1990. Growth of the slider turtle. Pp. 135–145. In J. W. Gibbons, Life History and Ecology of the Slider Turtle. Smithsonian Institution Press, Washington, DC.

Dunham, A. E., P. J. Morin, and H. M. Wilbur. 1988. Methods for the study of reptile populations. Pp. 331–386. In C. Gans and R. B. Huey (eds.), *Biology of the Reptilia,* Vol. 16. *Ecology B: Defense and Life History.* Alan R. Liss, New York.

Dunson, W. A. (ed.). 1975a. *The Biology of Sea Snakes*. University Park Press, Baltimore, Maryland.

Dunson, W. A. 1975b. Sea snakes of tropical Queensland between 18° and 20° south latitude. Pp. 151–162. In W. A. Dunson (ed.), *The Biology of Sea Snakes*. University Park Press, Baltimore, Maryland.

Dunson, W. A., and G. W. Ehlert. 1971. Effects of temperature, salinity, and surface water flow on distribution of the sea snake *Pelamis*. Limnology and Oceanography 16:845–853.

Dunson, W. A., and S. A. Minton. 1978. Diversity, distribution, and ecology of Philippine marine snakes (Reptilia, Serpentes). Journal of Herpetology 12:281–286.

Durtsche, R. D. 1996. A capture technique for small, smooth-scaled lizards. Herpetological Review 27:12–13.

Eberhardt, L. L., D. G. Chapman, and J. R. Gilbert. 1979. A review of marine mammal census methods. Wildlife Monographs 63:3–46.

Eckert, S. A. 1995. Telemetry and the behavior of sea turtles. Pp. 583–584. In K. A. Bjorndal (ed.), *Biology and Conservation of Sea Turtles*. Rev. ed. Smithsonian Institution Press, Washington, DC.

Eckert, S. A. 1999. Data acquisition systems for monitoring sea turtle behavior and physiology. Pp. 88–93. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, and M. Donnelly (eds.), *Research and Managements Techniques for the Conservation of Sea Turtles*. IUCN/SSC Marine Turtle Specialist Group Publication no. 4.

Edwards, D. 2000. Data quality assurance. Pp. 70–91. In W. K. Michener and J. Brunt (eds.), *Ecological Data: Design, Management, and Processing*. Blackwell Science, London, United Kingdom.

Efford, M. 2004. Density estimation in live-trapping studies. Oikos 106:598–610.

Efford, M. G., D. K. Dawson, and C. S. Robbins. 2004. DENSITY: Software for analyzing capture-recapture data from passive detector arrays. Animal Biodiversity and Conservation 27:217–228.

Efford, M. G., B. Warburton, M. C. Coleman, and R. J. Barker. 2005. A field test of two methods for density estimation. Wildlife Society Bulletin 33:731–738.

Ehmann, H., and H. Cogger. 1985. Australia's endangered herpetofauna: A review of criteria and policies. Pp. 435–447. In G. Grigg, R. Shine, and H. Ehmann (eds.), *Biology of Australasian Frogs and Reptiles*. Royal Zoological Society of New South Wales and Surrey Beatty, Shippping Norton, New South Wales, Australia.

Eidenmüller, B., and H.-D. Philippen. 2008. *Varanoid Lizards*. Chimaira, Frankfurt am Main, Germany.

Elbroch, M. 2003. *Mammal Tracks and Sign: A Guide to North American Species*. Stackpole, Mechanicsburg, Pennsylvania.

Elsey, R. M., and C. S. Wink. 1986. The effects of estradiol on plasma calcium and femoral bone structure in alligators (*Alligator mississippiensis*). Comparative Biochemistry and Physiology 84A:107–110.

Elzinga, C. L., D. W. Salzer, J. W. Willoughby, and J. P. Gibbs. 2001. *Monitoring Plant and Animal Populations: A Handbook for Field Biologists*. Blackwell Science, Malden, Massachusetts.

Emmons, L. H. 1989. Jaguar predation on chelonians. Journal of Herpetology 23:311–314.

Enge, K. M. 1997a. *A Standardized Protocol for Drift-fence Surveys*. Florida Game and Freshwater Fish Commission Nongame Technical Report no.14, Tallahassee, Florida.

Enge, K. M. 1997b. Use of silt fencing and funnel traps for drift fences. Herpetological Review 28:30–31.

Enge, K. M. 2001. The pitfalls of pitfall traps. Journal of Herpetology 35:467–478.

Engelstoft, C., and K. E. Ovaska. 2000. Artificial cover-objects as a method for sampling snakes (*Contia tenuis* and *Thamnophis* spp.) in British Columbia. Northwestern Naturalist 81:35–43.

Engelstoft, C., K. Ovaska, and N. Honkanen. 1999. The harmonic direction finder: A new method for tracking movements of small snakes. Herpetological Review 30:84–87.

Engeman, R. M. 2003. More on the need to the get the basics right: Population indices. Wildlife Society Bulletin 31:286–287.

Engstrom, T. N., H. B. Shaffer, and W. P McCord. 2002. Phylogenetic diversity of endangered and critically endangered southeast Asian softshell turtles (Trionychidae: *Chitra*). Biological Conservation 104:173–179.

Ennion, E. A. R., and N. Tinbergen. 1967. *Tracks*. Clarendon, Oxford University Press, London, England.

Environmental Systems Research Inc. (ESRI). 1999. *Getting to Know ArcView GIS Self-Study Workbook*. ESRI Press, Redlands, California.

Epperly, S. P., J. Braun, and A. J. Chester. 1995. Aerial surveys for sea turtles in North Carolina inshore waters. Fishery Bulletin 93:254–261.

Epperly, S. P., J. Braun, A. J. Chester, F. A. Cross, J. V. Merriner, P. A. Tester, and W. T. Hogarth. 1995. Distribution and abundance of sea turtles in North Carolina coastal waters in winter months. Pp. 28–30. In J. I. Richardson and T. H. Richardson (compilers), *Proceedings of the Twelfth Annual Workshop on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFC-361. National Marine Fisheries Service, Miami, Florida.

Epperly, S. P., A. Veishlow, and J. Braun. 1991. Distribution and species composition of sea turtles in North Carolina, 1989–1990. Pp. 155–157. In M. Salmon and J. Wyneken (compilers), *Proceedings of the Eleventh Annual Workshop on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFSC-302. National Marine Fisheries Service, Miami, Florida.

Erickson, G. M. 2003. Using external vertebral growth rings to assess longevity in the Bengal Monitor (*Varanus bengalensis*). Copeia 2003:872–878.

Ernst, C. H. 1976. Ecology of the Spotted Turtle, *Clemmys guttata* (Reptilia, Testudines, Testudinidae), in southeastern Pennsylvania. Journal of Herpetology 10:25–33.

Ernst, C. H., and R. W. Barbour. 1989. *Turtles of the World*. Smithsonian Institution Press, Washington, DC.

Ernst, C. H., W. A. Cox, and K. R. Marion. 1983. The Distribution and Status of the Flattened Musk Turtle in the Warrior Basin of Alabama. Unpubl. Contract Report, Alabama Coal Association, Mountain Brook, Alabama.

Ernst, C. H., W. A. Cox, and K. R. Marion. 1989. The distribution and status of the Flattened Musk Turtle, *Sternotherus depressus* (Testudines: Kinosternidae). Tulane Studies in Zoology and Botany 27:1–20.

Ernst, C. H., M. F. Hershey, and R. W. Barbour. 1974. A new coding system for hardshelled turtles. Transactions of the Kentucky Academy of Science 35:27–28.

Ernst, C. H., and J. E. Lovich. 2009. *Turtles of the United States and Canada*. 2nd ed. Johns Hopkins University Press, Baltimore, Maryland.

Ernst, C. H., R. T. Zappalorti, and J. E. Lovich. 1989. Overwintering sites and thermal relations of hibernating Bog Turtles, *Clemmys muhlenbergii*. Copeia 1989:761–764.

Erwin, T. L. 1997. Biodiversity at its utmost: Tropical forest beetles. Pp. 27–40. In M. L. Reaka-Kudla, D. E. Wilson, and E. O. Wilson (eds.), *Biodiversity II. Understanding and Protecting our Biological Resources*. Joseph Henry, Washington, DC.

Escobedo Galván, A. H., M. Venegas-Anaya, M. R. Espinal, S. G. Platt, and F. Buitrago. 2010. Conservation of Crocodilians in Mesoamerica.

Pp. 746–757. In L. D. Wilson, J. H. Townsend, and J. D. Johnson (eds.), *Conservation of Mesoamerican Amphibians and Reptiles*. Eagle Mountain Publishing, Eagle Mountain, Utah.

Estes, R., and G. Pregill (eds.). 1988. *Phylogenetic Relationships of Lizard Families. Essays Commemorating Charles L. Camp*. Stanford University Press, Stanford, California.

Etheridge, K., and L. C. Wit. 1993. Factors affecting activity in *Cnemidophorus*. Pp. 151–162. In J. W. Wright and L. J. Vitt (eds.), *Biology of Whiptail Lizards (Genus* Cnemidophorus*)*. Oklahoma Museum of Natural History and University of Oklahoma, Norman, Oklahoma. Herpetologists' League Special Publication no. 3.

Eubanks, J. O., J. W. Hollister, C. Guyer, and W. K. Michener. 2002. Reserve area requirements for Gopher Tortoises (*Gopherus polyphemus*). Chelonian Conservation and Biology 4:464–471.

Fabrezi, M., A. Manzano, V. Abdala, and H. Zaher. 2009. Developmental basis of limb homology in leurodiran turtles, and the identity of the hooked element in the chelonian tarsus. Zoological Journal of the Linnaean Society 155:845–866.

Faccio, S. D. 2001. *Biological Inventory of Amphibians and Reptiles at the Marsh-Billings-Rockefeller National Historical Park and Adjacent Lands*. National Park Service, Northeast Region, Technical Report NPS/NER/NRTR—2005/008, Woodstock, Vermont. http://science.nature.nps.gov/im/units/netn/downloads/MABI_herp_TIC_d26.pdf

Fager, E. W. 1972. Diversity: A sampling study. American Naturalist 106:293–310.

Fahrig, L., J. H. Pedlar, S. E. Pope, P. D. Taylor, and J. F. Wegner. 1995. Effect of road traffic on amphibian density. Biological Conservation 73:177–182.

Fair, W. S., and S. E. Henke. 1997a. Efficacy of capture methods for a low density population of *Phrynosoma cornutum*. Herpetological Review 28:135–137.

Fair, W. S., and S. E. Henke. 1997b. Effects of habitat manipulations on Texas Horned Lizards and their prey. Journal of Wildlife Management. 61:1366–1370.

Faith, D. P. 1992a. Conservation evaluation and phylogenetic diversity. Biological Conservation 61:1–10.

Faith, D. P. 1992b. Systematics and conservation: On predicting the feature diversity of subsets of taxa. Cladistics 8:361–373.

Faith, D. P. 1994. Phylogenetic pattern and the quantification of organismal biodiversity. Philosophical Transactions of the Royal Society London B 345:45–58.

Farallo, V. R., D. J. Brown, and M. R. J. Forstner. 2010. An improved funnel trap for drift-fence surveys. Southwestern Naturalist 55:457–460.

Farr, D. R., and P. T. Gregory. 1991. Sources of variation in estimating litter characteristics of the garter snake, *Thamnophis elegans*. Journal of Herpetology 25:261–268.

Fauth, J. E., B. I. Crother, and J. B. Slowinski. 1989. Elevational patterns of species richness, evenness, and abundance of the Costa Rican leaf-litter herpetofauna. Biotropica 21:178–185.

Fellers, G. M. 1997. Design of amphibian surveys. Pp. 23–34. In D. H. Olson, W. P. Leonard, and R. B. Bury (eds.), *Sampling Amphibians in Lentic Habitats: Methods and Approaches for the Pacific Northwest*. Society for Northwestern Vertebrate Biology, Northwest Fauna no. 4.

Fellers, G. M., and C. A. Drost. 1989. Fluorescent powder—a method for tracking reptiles. Herpetological Review 20:91–92.

Fellers, G. M., and C. A. Drost. 1994. Sampling with artificial cover. Pp. 146–150. In W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster (eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Smithsonian Institution Press, Washington, DC.

Fellers, G. M., and K. L. Freel. 1995. *A Standardized Protocol for Surveying Aquatic Amphibians*. U.S. Department of the Interior, National Park Service, Western Region, University of California, Davis, California. Technical Report NPS/WRUC/NRTR-95-01.

Fellers, G. M., and D. Pratt. 2002. *Terrestrial Vertebrate Inventory, Point Reyes National Seashore, 1998–2001*. Prepared for National Park Service. USGS Western Ecological Research Center, Sacramento, California. http://www.werc.usgs.gov/ProductsSearch.aspx

Ferguson, A. W., F. W. Weckerly, J. T. Baccus, and M. R. J. Forstner. 2008. Evaluation of predator attendance at pitfall traps in Texas. Southwestern Naturalist 53:450–457.

Ferguson, G. W. 1976. Color change and reproductive cycling in female Collared Lizards (*Crotaphytus collaris*). Copeia 1976:491–494.

Ferguson, M. J. W. 1985. Reproductive biology and embryology of the crocodilians. Pp. 329–491. In C. Gans, G. Billett, and P. F. A. Maderson (eds.), *Biology of the Reptilia,* Vol. 14. *Development A*. Wiley, New York.

Ferner, J. W. 2007. *A Review of Marking Techniques for Amphibians and Reptiles*. Society for the Study of Amphibians and Reptiles, Herpetological Circular 35.

Ferner, J. W., R. M. Brown, R. V. Sison, and R. S. Kennedy. 2001. The amphibians and reptiles of Panay island, Philippines. Asiatic *Herpetological Research* 9:34–70.

Fields, J. R., T. R. Simpson, R. W. Manning, and R. L. Rose. 2000. Modifications of the stomach flushing technique for turtles. Herpetological Review 31:32–33.

Finklestein, P. L., J. C. Kaimal, J. E. Gaynor, M. E. Graves, and T. J. Lockhart. 1986. Comparison of wind monitoring systems. Pt. I: In situ sensors. Journal of Atmospheric and Oceanic Technology 3:583–593.

Fisher, M., and A. Muth. 1989. A technique for permanently marking lizards. Herpetological Review 20:45–46.

Fisher, M., and A. Muth. 1995. A backpack method for mounting radio transmitters to small lizards. Herpetological Review 26:139–140.

Fisher M. C., and T. W. J. Garner. 2007. The relationship between the emergence of *Batrachochytrium dendrobatidis,* the international trade in amphibians and introduced amphibian species. Fungal Biology Reviews 21:2–9.

Fisher, R. N., and T. J. Case. 2000. Distribution of the herpetofauna of coastal southern California with reference to elevation effects. Pp. 137–143. In J. E. Keeley, M. Baer-Keeley, and C. J. Fotheringham (eds.), *2nd Interface between Ecology and Land Development in California*. U.S. Geological Survey Open-file Report 00-62. http://pubs.usgs.gov/of/2000/of00-062/

Fisher, R., D. Stokes, C. Rochester, C. Brehme, S. Hathaway, and T. Case. 2008. *Herpetological Monitoring Using a Pitfall Trapping Design in Southern California*. U.S. Geological Survey Techniques and Methods 2-A5. http://pubs.usgs.gov/tm/tm2a5

Fisher, R. N., A. V. Suarez, and T. J. Case. 2002. Spatial patterns in the abundance of the Coastal Horned Lizard. Conservation Biology 16:205–215.

Fitch, H. S. 1949. Road counts of snakes in western Louisiana. Herpetologica 5:87–90.

Fitch, H. S. 1951. A simplified type of funnel trap for reptiles. Herpetologica 7:77–80.

Fitch, H. S. 1960. Criteria for determining sex and breeding maturity in snakes. Herpetologica 16:49–51.

Fitch, H. S. 1965. An ecological study of the garter snake, *Thamnophis sirtalis*. University of Kansas Publications, Museum of Natural History 15:493–564.

Fitch, H. S. 1987. Collecting and life-history techniques. Pp. 143–164. In R. A. Seigel, J. T. Collins, and S. S. Novak (eds.), *Snakes: Ecology and Evolutionary Biology*. Macmillan, New York.

Fitch, H. S. 1992. Methods of sampling snake populations and their relative success. Herpetological Review 23:17–19.

Fitch, H. S. 1999. *A Kansas Snake Community: Composition and Changes over 50 Years*. Krieger, Malabar, Florida.

Fitch, H. S., and R. W. Henderson. 1977. Age and sex differences, reproduction and conservation of *Iguana iguana*. Contributions in Biology and Geology, Milwaukee Public Museum 13:1–21.

Fitch, H. S., and M. V. Plummer. 1975. A preliminary ecological study of the soft-shelled turtle *Trionyx muticus* in the Kansas River. Israel Journal of Zoology 24:28–42.

Fitzgerald, L. A. 1990. The implications of hunting patterns and the utility of harvest monitoring for managing *Tupinambis* lizards. In *Technical Report Proyecto* Tupinambis *CITES/WWF/CICUR*. World Wildlife Fund, Washington, DC. http://herpetology.tamu.edu/Fitzgerald/Publications.htm

Fitzgerald, L. A. 1994a. The interplay between life history and environmental stochasticity: Implications for the management of exploited lizard populations. American Zoologist 34:371–381.

Fitzgerald, L. A. 1994b. *Tupinambis* lizards and people: A sustainable use approach to conservation and development. Conservation Biology 8:12–16.

Fitzgerald, L. A., J. M. Chani, and O. E. Donadío. 1991. *Tupinambis* lizards in Argentina: Implementing management of a traditionally exploited resource. Pp. 303–316. In J. G. Robinson and K. H. Redford (eds.), *Neotropical Wildlife Use and Conservation*. University of Chicago Press, Chicago, Illinois.

Fitzgerald, L. A., F. B. Cruz, and G. Perotti. 1993. The reproductive cycle and size at maturity of *Tupinambis rufescens* (Sauria: Teiidae) in the dry chaco of Argentina. Journal of Herpetology 27:70–78.

Fitzgerald, L. A., F. B. Cruz, and G. Perotti. 1999. Phenology of a lizard assemblage in the dry chaco of Argentina. Journal of Herpetology 33:526–535.

Fitzgerald, L. A., and C. W. Painter. 2000. Rattlesnake commercialization: Long-term trends, issues, and implications for conservation. Wildlife Society Bulletin 28:235–253.

Fitzgerald, L. A., C. W. Painter, A. Reuter, and C. Hoover. 2004. *Collection, Trade, and Regulation of Reptiles and Amphibians of the Chihuahuan Desert Ecoregion*. TRAFFIC North America, World Wildlife Fund, Washington, DC. http://www.traffic.org/search-publications/

Fitzgerald, L. A., G. Porini, and V. Lichtschein. 1994. El manejo de *Tupinambis* en Argentina: Historia, estado actual y perspectivas futuras. Interciencia 19:166–170.

Fitzgerald, S. 1989. International wildlife trade: Whose business is it? World Wildlife Fund, Washington, DC.

FitzSimmons, N., C. Moritz, and B. W. Bowen. 1999. Population identification. Pp. 72–79. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, and M. Donnelly (eds.), *Research and Management Techniques for the Conservation of Sea Turtles*. IUCN/SSC Marine Turtle Specialist Group Publication no. 4.

Flores-Villela, O. A., and G. R. Zug. 1995. Reproductive biology of the Chopontil, *Claudius angustatus* (Testudines: Kinosternidae), in southern Veracruz, México. Chelonian Conservation and Biology 1:181–186.

Florida Fish and Wildlife Conservation Commission. 2007. *Marine Turtle Conservation Guidelines*. Tallahassee, Florida. http://myfwc.com/media/418106/Seaturtle_Guidelines.pdf

Font, E., and J. M. Schwartz. 1989. Ketamine as an anesthetic for some squamate reptiles. Copeia 1989:484–486.

Fontaine, C. T., T. D. Williams, and J. D. Camper. 1987. Ridleys tagged with passive integrated transponder (PIT). Marine Turtle Newsletter 41:6.

Ford, N. B., and P. M. Hampton. 2005. The amphibians and reptiles of Camp Maxey, Lamar County, Texas with comments on census methods. Texas Journal of Science 57:359–370.

Forey, P. L., C. J. Humphries, and R. I. Vane-Wright (eds.). 1994. *Systematics and Conservation Evaluation*. Systematics Association Special Volume 50. Clarendon, Oxford University Press, Oxford, England.

Forney, K. A., D. A. Hanan, and J. Barlow. 1991. Detecting trends in harbor porpoise abundance from aerial surveys using analysis of covariance. Fishery Bulletin 89:367–377.

Forsman, A., and L. E. Lindell. 1996. Resource dependent growth and body condition dynamics in juvenile snakes: An experiment. Oecologia 108:669–675.

Fortin, M.-J., and M. R. T. Dale. 2005. *Spatial Analysis: A Guide for Ecologists*. Cambridge University Press, Cambridge, Massachusetts.

Foster, M. S. 1982. The research natural history museum: Pertinent or passé? Biologist 64:1–12.

Foster, M. S., and L. A. Fitzgerald. 1982. A technique for live-trapping cormorants. Journal of Field Ornithology 53:422–423.

Fox, H. 1977. The urinogenital system of reptiles. Pp.1–157. In C. Gans and T. S. Parsons (eds.), *Biology of the Reptilia,* Vol. 6. *Morphology E*. Academic Press, New York.

Fowler, M. E. 1995. *Restraint and Handling of Wild and Domestic Animals*. 2nd ed. Iowa State University Press, Ames, Iowa.

Francis, R. I. C. C. 1990. Back-calculation of fish length: A critical review. Journal of Fish Biology 36:883–902.

Franke, J., and T. M. Telecky. 2001. *Reptiles as Pets: An Examination of the Trade in Live Reptiles in the United States*. Humane Society of the United States, Washington, DC.

Franklin, I. R. 1980. Evolutionary changes in small populations. Pp. 135–149. In M. E. Soulé and B. A. Wilcox (eds.), *Conservation Biology: An evolutionary-ecological perspective*. Sinauer, Sunderland, Massachusetts.

Franklin, J., K. Wejnert, S. A. Hathaway, C. J. Rochester, and R. N. Fisher. 2009. Effect of species rarity on the accuracy of species distribution models for reptiles and amphibians in southern California. Diversity and Distributions 15:167–177.

Frazer, N. B., and H. J. Frith. 1973. *Wildlife Conservation*. Angus Robertson, Sydney, Australia.

Frazer, N. B., J. W. Gibbons, and T. J. Owens. 1990. Turtle trapping: Preliminary tests of conventional wisdom. Copeia 1990:1150–1152.

Frazer, N. B., J. L. Greene, and J. W. Gibbons. 1993. Temporal variation in growth rate and age at maturity of male Painted Turtles, *Chrysemys picta*. American Midland Naturalist 130: 314–324.

Freed, P. S., and M. G. Freed. 1983. An additional restraint technique for venomous snakes. Herpetological Review 14:114.

Freilich, J. E., and E. L. LaRue, Jr. 1998. Importance of observer experience in finding desert tortoises. Journal of Wildlife Management 62:590–596.

Fricke, H. W. 1970. Die ökologische spezialisierung der eidechse *Cryptoblepharus boutoni cognatus* (Boetteger) auf das leben in der gezeitenzone (Reptilia, Skinkidae). Oecologia 5:380–391.

Frith, H. J. 1973. *Wildlife Conservation*. Angus Robertson, Sydney, Australia.

Fritschen, L. J., and L. W. Gay. 1979. *Environmental Instrumentation*. Springer, New York.

Fritts, T. H. 1981. Pelagic feeding habits of turtles in the Eastern Pacific. Marine Turtle Newsletter 17:4–5.

Fritts, T. H. 1982. Plastic bags in the intestinal tracts of leatherback marine turtles. Herpetological Review 13:72–73.

Fritts, T. H. 1988. The Brown Tree Snake, *Boiga irregularis,* a threat to Pacific islands. U.S. Fish and Wildlife Service, Biological Report 88(31), 36 pp.

Fritts, T. H., A. B. Irvine, R. D. Jennings, L. A. Collum, W. Hoffman, and M. A. McGehee. 1983. *Turtles, Birds, and Mammals in the Northern Gulf of Mexico and Nearby Atlantic Waters*. US Fish and Wildlife Service, Division of Biological Services, FWS/OBS-82/65.

Fry, G. C., D. A. Milton, and T. J. Wassenberg. 2001. The reproductive biology and diet of sea snake bycatch of prawn trawling in Northern Australia: Characteristics important for assessing the impacts on populations. Pacific Conservation Biology 7:55–73.

Frye, F. L. 1991. *Biomedical and Surgical Aspects of Captive Reptile Husbandry*. 2 vol. 2nd ed. Krieger, Malabar, Florida.

Fukuda, Y., G. Webb, C. Manolis, R. Delaney, M. Letnic, G. Lindner, and P. Whitehead. 2011. Recovery of saltwater crocodiles following unregulated hunting in tidal rivers of the Northern Territory, Australia. Journal of Wildlife Management 75:1253–1266.

Fuller, D. A., and R. Lohoefener. 1990. Sea turtles on the Chandeleur and Breton Islands, Louisiana. Pp. 35–38. In T. H. Richardson, J. I. Richardson, and M. Donnelly (compilers), *Proceedings of the Tenth Annual Workshop on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFC-278. National Marine Fisheries Service, Miami, Florida.

Fuller, W. A. 1996. *Introduction to Statistical Time Series*. 2nd ed. Wiley, New York.

Funk, V. A., P. C. Hoch, L. A. Prather, and W. L. Wagner. 2005. The importance of vouchers. Taxon 54:127–129.

Furilla, R. A., and D. Bartlett, Jr. 1989. Intrapulmonary CO_2 inhibits inspiration in garter snakes. Respiration Physiology 78:207–218.

Gabe, M., and H. Saint Girons. 1964. Contribution a l'histologie de *Sphenodon punctatus* Gray. Centre National de la Recherché Scientifique, Paris, France.

Galán, P. 1999. Demography and population dynamics of the lacertid lizard *Podarcis bocagei* in north-west Spain. Journal of Zoology, London 249:203–218.

Galbraith, D. A., and R. J. Brooks. 1983. A simple restraining device for large turtles. Herpetological Review 14:115.

Galbraith, D. A., and R. J. Brooks. 1987a. Photographs and dental casts as permanent records for age estimates and growth studies of turtles. Herpetological Review 18:69–71.

Galbraith, D. A., and R. J. Brooks. 1987b. Addition of annual growth lines in adult Snapping Turtles *Chelydra serpentina*. Journal of Herpetology 21:359–363.

Galligan, J. H., and W. A. Dunson. 1979. Biology and status of Timber Rattlesnake (*Crotalus horridus*) populations in Pennsylvania. Biological Conservation 15:13–58.

Gambold, N., and J. C. Z. Woinarski. 1993. Distributional patterns of herpetofauna in monsoon rainforests of the Northern Territory, Australia. Australian Journal of Ecology 18:431–449.

Games, I. 1990. Growth curves for the Nile crocodile as estimated by skeletochronology. Pp. 11–121. In *Crocodiles: Proceedings of the 10th Working Meeting of the Crocodile Specialist Group of the Species Survival Commission of IUCN - The World Conservation Union*. IUCN - The World Conservation Union, Gland, Switzerland.

Gans, C., and A. M. Taub. 1964. Precautions for keeping poisonous snakes in captivity. Curator 7:196–205.

Garber, S. D., and J. Burger. 1995. A 20-yr study documenting the relationship between turtle decline and human recreation. Ecological Applications 5:1151–1162.

Gardner, A. S. 1984. The Evolutionary Ecology and Population Systematics of Day Geckos (*Phelsuma*) in the Seychelles. Unpubl. Ph.D. dissert., University of Aberdeen, Aberdeen, United Kingdom.

Gardner, S., E. H. W. Baard, and N. J. le Roux. 1999. Estimating the detection probability of the Geometric Tortoise. South African Journal of Wildlife Research 29:62–71.

Gardner, S. L. 1996. Field parasitology techniques for use with mammals. Pp. 291–298. In D. E. Wilson, F. R. Cole, J. D. Nichols, R. Rudran, and M. S. Foster (eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Mammals*. Smithsonian Institution Press, Washington, DC.

Garland, T., Jr., and W. G. Bradley. 1984. Effects of a highway on Mojave Desert rodent populations. American Midland Naturalist 111:47–56.

Garner, G. W., S. C. Amstrup, J. L. Laake, B. F. J. Manly, L. L. McDonald, and D. G. Robertson (eds.). 1999. *Marine Mammal Survey and Assessment Methods*. Balkema, Rotterdam, The Netherlands.

Gasc, J-P., A. Cabela, J. Crnobrnja-Isailovic., D. Dolmen, K. Grossenbacher, P. Haffner, J. Lescure, H. Martens, J. P. Martínez Rica, H. Maurin, M. E. Oliveira, T. S. Sofianidou, M. Veith, and A. Zuiderwijk (eds.). 1997. *Atlas of Amphibians and Reptiles in Europe*. Patrimoines naturels 29. Societas Europaea Herpetologica and Muséum National d'Histoire Naturelle, Paris, France. [Reissue 2004.]

Gaston, K. J. 1996. What is biodiversity? Pp. 1–9. In K. J. Gaston (ed.), *Biodiversity: A Biology of Numbers and Difference*. Blackwell Science, Cambridge, Massachusetts.

Gaston, K. J., and P. H. Williams. 1996. Spatial patterns in taxonomic diversity. Pp. 202–229. In K. J. Gaston (ed.), *Biodiversity: A Biology of Numbers and Difference*. Blackwell Science, Cambridge, Massachusetts.

Gates, C. E. 1979. Line transect and related issues in sampling biological populations. Pp. 71–154. In R. M. Cormack, G. P. Patil, and D. S. Robson (eds.), *Sampling Biological Populations*. Second International Congress of Ecology, Hebrew University, Jerusalem, Israel 1978. International Cooperative Publishing House, Fairland, Maryland.

Gaulke, M. 1997. Sex determination of monitor lizards in the field - A review of methods. Hamadryad 22:28–31.

Gaulke, M., W. Erdelen, and F. Abel. 1999. A radio-telemetric study of the Water Monitor Lizard (*Varanus salvator*) in North Sumatra, Indonesia. Pp. 63–78. In H. G. Horn and W. Bohme (eds.), *Advances in Monitor Research II*. Mertensiella 11.

Georges, A. 1985. Reproduction and reduced body size of reptiles in unproductive insular environments. Pp. 311–318. In G. Grigg, R. Shine, and H. Ehmann (eds.), *Biology of Australasian Frogs and Reptiles*. Royal Zoological Society of New South Wales and Surrey Beatty, Shippping Norton, New South Wales, Australia.

Georges, A. 1997. Modelling incubation period and constant temperature equivalence for reptile embryos incubated under fluctuating temperature regimes. P. 139. In *American Society of Ichthyologists and Herpetologists 77th Annual Meeting, University of Washington, Seattle, Washington, Programs and Abstracts*.

Germano, D. J. 1994. Growth and age at maturity of North American tortoises in relation to regional climates. Canadian Journal of Zoology 72:918–931.

Germano, D. J., and R. B. Bury. 1998. Age determination in turtles: Evidence of annual deposition of scute rings. Chelonian Conservation and Biology 3:123–132.

Gerrodette, T. 1987. A power analysis for detecting trends. Ecology 68:1364–1372.

Gerrodette, T. 1993. TRENDS: Software for a power analysis of linear regression. Wildlife Society Bulletin 21:515–516.

Gerrodette, T. 2000. Estimating abundance with transects. Pp. 10–15. In K. A. Bjorndal and A. B. Bolten (eds.), *Proceedings of a Workshop on Assessing Abundance and Trends for In-Water Sea Turtle Populations*. NOAA Technical Memorandum NMFS-SEFSC-445. National Marine Fisheries Service, Miami, Florida.

Gerrodette, T., and J. Brandon. 2000. Designing a monitoring program to detect trends. Pp. 36–39. In K. A. Bjorndal and A. B. Bolten (eds.), *Proceedings of a Workshop on Assessing Abundance and Trends for In-Water Sea Turtle Populations*. NOAA Technical Memorandum NMFS-SEFSC-445. National Marine Fisheries Service, Miami, Florida.

Gerrodette, T., and B. L. Taylor. 1999. Estimating population size. Pp. 67–71. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, and M. Donnelly (eds.), *Research and Management Techniques for the Conservation of Sea Turtles*. IUCN/SSC Marine Turtle Specialist Group Publication no. 4.

Getz, W. M., and R. G. Haight. 1989. *Population Harvesting: Demographic Models of Fish, Forest, and Animal Resources*. Princeton University Press, Princeton, New Jersey.

Gibbons, J. W. 1968a. Observations on the ecology and population dynamics of the Blanding's turtle, *Emydoidea blandingi*. Canadian Journal of Zoology 46:288–290.

Gibbons, J. W. 1968b. Population structure and survivorship in the Painted Turtle, *Chrysemys picta*. Copeia 1968:260–268.

Gibbons, J. W. 1970. Terrestrial activity and the population dynamics of aquatic turtles. American Midland Naturalist 83:404–414.

Gibbons, J. W. 1972. Reproduction, growth, and sexual dimorphism in the Canebrake Rattlesnake (*Crotalus horridus atricaudatus*). Copeia 1972:222–226.

Gibbons, J. W. 1976. Aging phenomena in reptiles. Pp. 453–475. In M. F. Elias, B. E. Eleftheriou, and P. K. Elias (eds.), *Special Review of Experimental Aging Research*. Environmental Aging Research, Bar Harbor, Maine.

Gibbons, J. W. 1990. Turtle studies at SREL: A research perspective. Pp. 19–44. In J. W. Gibbons, *Life History and Ecology of the Slider Turtle*. Smithsonian Institution Press, Washington, DC.

Gibbons, J. W., and K. M. Andrews. 2004. PIT tagging: Simple technology at its best. BioScience 54:447–454.

Gibbons, J. W., and D. H. Bennett. 1974. Determination of anuran terrestrial activity patterns by a drift fence method. Copeia 1974:236–243.

Gibbons, J. W., J. W. Coker, and T. M. Murphy, Jr. 1977. Selected aspects of the life history of the Rainbow Snake (*Farancia erytrogramma*). Herpetologica 33:276–281.

Gibbons, J. W., and M. E. Dorcas. 2004. *North American Watersnakes: A Natural History*. University of Oklahoma Press, Norman, Oklahoma.

Gibbons, J. W., and M. Dorcas. 2005. *Snakes of the Southeast*. University of Georgia Press, Athens, Georgia.

Gibbons, J. W., and J. L. Greene. 1979. X-ray photography: A technique to determine reproductive patterns of freshwater turtles. Herpetologica 35:86–89.

Gibbons, J. W., G. H. Keaton, J. P. Schubauer, J. L. Greene, D. H. Bennett, J. R. McAuliffe, and R. R. Sharitz. 1979. Unusual population size structure in freshwater turtles on barrier islands. Georgia Journal of Science 37:155–159.

Gibbons, J. W., J. E. Lovich, A. D. Tucker, N. N. FitzSimmons, and J. L. Greene. 2001. Demographic and ecological factors affecting conservation and management of the Diamondback Terrapin (*Malaclemys terrapin*) in South Carolina. Chelonian Conservation and Biology 4:66–74.

Gibbons, J. W., D. E. Scott, T. J. Ryan, K. A. Buhlmann, T. D. Tuberville, B. S. Metts, J. L. Greene, T. Mills, Y. Leiden, S. Poppy, and C. T. Winne. 2000. The global decline of reptiles, déjà vu amphibians. Bioscience 50:653–666.

Gibbons, J. W., and R. D. Semlitsch. 1982. Terrestrial drift fences with pitfall traps: An effective technique for quantitative sampling of animal populations. Brimleyana 7:1–16.

Gibbons, J. W., and R. D. Semlitsch. 1987. Activity patterns. Pp. 396–421. In R. A. Seigel, J. T. Collins, and S. S. Novak (eds.), *Snakes: Ecology and Evolutionary Biology*. Macmillan, New York.

Gibbons, W. 1983. *Their Blood Runs Cold: Adventures with Reptiles and Amphibians*. University of Alabama Press, University, Alabama.

Gibbons, W., J. Greene, and T. Mills. 2009. *Lizards and Crocodilians of the Southeast*. University of Georgia Press, Athens, Georgia.

Gibbs, J. P., S. Droege, and P. Eagle. 1998. Monitoring populations of plants and animals. BioScience 48:935–940.

Gienger, C. M., D. D. Beck, N. C. Sabari, and D. L. Stumbaugh. 2002. Dry season habitat use by lizards in a tropical deciduous forest of western Mexico. Journal of Herpetology 36:487–490.

Gilbert, S. F., and D. Epel. 2009. *Ecological Developmental Biology: Integrating Epigenetics, Medicine, and Evolution*. Sinauer, Sunderland, Massachusetts.

Gilbert, S. F., G. A. Loredo, A. Brukman, and A. C. Burke. 2001. Morphogenesis of the turtle shell: The development of a novel structure in tetrapod evolution. Evolution and Development 3:47–58.

Gill, D. E. 1978. The metapopulation ecology of the Red-spotted Newt, *Notophthalmus viridescens* (Rafinesque). Ecological Monographs 48:145–166.

Gillingham, J. C., and C. C. Carpenter. 1978. Snake hibernation: Construction of and observations on a man-made hibernaculum (Reptilia, Serpentes). Journal of Herpetology 12:495–498.

Gillingham, J. C., D. L. Clark, and G. R. Ten Eyck. 1983. Venomous snake immobilization: A new technique. Herpetological Review 14:40.

Gilman, C. A., and B. O. Wolf. 2007. Use of portable ultrasonography as a nondestructive method for estimating reproductive effort in lizards. Journal of Experimental Biology 210:1859–1867.

Giraudo, A. R. 2001. *Serpientes de la Selva Paranense y del Chaco Húmedo.* Literature of Latin America (L. O. L. A.), Buenos Aires, Argentina.

Glaw, F., and M. Vences. 2007. *A Field Guide to the Amphibians and Reptiles of Madagascar.* 3rd ed. Vences Glaw, Cologne, Germany.

Glenn, J. L., R. Straight, and C. C. Snyder. 1972. Clinical use of ketamine hydrochloride as an anesthetic agent for snakes. American Journal of Veterinary Research 33:1901–1903.

Glor, R. E., A. S. Flecker, M. F. Bernard, and A. G. Power. 2001. Lizard diversity and agricultural disturbance in a Caribbean forest landscape. Biodiversity and Conservation 10:711–723.

Glor, R. E., T. M. Townsend, M. F. Benard, and A. S. Flecker. 2000. Sampling reptile diversity in the West Indies with mouse glue-traps. Herpetological Review 31:88–90.

Gloyd, H. K. 1946. Some rattlesnake dens of South Dakota. Chicago Naturalist 9:87–97.

Goater, T. M., and C. P. Goater. 2001. *Ecological Monitoring and Assessment Network (EMAN) Protocols for Measuring Biodiversity: Parasites of Amphibians and Reptiles.* 59 pp. Environment Canada, Ecological Monitoring and Assessment Network: http://www.ec.gc.ca/faunescience-wildlifescience/default.asp?lang=En&n=9C4F74C5-1 [see: wildlife and landscape>monitoring>ecological monitoring>terrestrial ecosystems]

Godley, J. S. 1980. Foraging ecology of the Striped Swamp Snake *Regina alleni,* in southern Florida. Ecological Monographs 50:411–436.

Godley, J. S. 1982. Aquatic snakes in water hyacinth communities. Pp. 281–282. In D. E. Davis (ed.), *CRC Handbook of Census Methods for Terrestrial Vertebrates.* CRC Press, Boca Raton, Florida.

Goin, C. J. 1942. A method for collecting the vertebrates associated with water hyacinths. Copeia 3:183–184.

Goin, C. J. 1943. The lower vertebrate fauna of the water hyacinth community in northern Florida. *Proceedings of the Florida Academy of Sciences* 6:143–154.

Goldberg, S. R., and C. R. Bursey. 1991. *Parapharyngodon kartana* in two skinks, *Emoia nigra* and *Emoia samoense* (Sauria: Scincidae), from Samoa. Journal of the Helminthological Society of Washington 58:265–266.

Goldberg, S. R., and C. R. Bursey. 2000. Transport of helminthes to Hawaii via the brown anole, *Anolis sagrei* (Polychrotidae). Journal of Parasitology 86:750–755.

Goldberg, S. R., and C. R. Bursey. 2002. Gastrointestinal helminths of seven gekkonid lizard species (Sauria: Gekkonidae) from Oceania. Journal of Natural History 36:2249–2264.

Goldberg, S. R., C. R. Bursey, and R. N. Fisher. 2005. Helminth Records from Eleven Species of *Emoia* (Sauria: Scincidae) from Oceania. Pacific Science 59:609–614.

Goldingay, R. L., and D. A. Newell. 2000. Experimental rock outcrops reveal continuing habitat disturbance for an endangered Australian snake. Conservation Biology 14:1908–1912.

Gomez, C., E. M. Özbudak, J. Wunderlich, D. Baumann, J. Lewis, and O. Pourquié. 2008. Control of segment number in vertebrate embryos. Nature 454:335–339.

Goode, J. 1967. *Freshwater Tortoises of Australia and New Guinea (in the family Chelidae).* Lansdowne, Melbourne, Australia.

Goode, M. J., W. C. Horrace, M. J. Sredl, and J. M. Howland. 2005. Habitat destruction by collectors associated with decreased abundance of rock-dwelling lizards. Biological Conservation 125:47–54.

Goode, M. J., D. E. Swann, and C. R. Schwalbe. 2004. Effects of destructive collecting practices on reptiles: A field experiment. Journal of Wildlife Management 68:429–434.

Goodman, S. M., and J. P. Benstead (eds.). 2003. *The Natural History of Madagascar.* University of Chicago Press, Chicago, Illinois.

Goovaerts, P. 1997. *Geostatisics for Natural Resources Evaluation.* Oxford University Press, New York.

Gorman, G. C. 1985. Reproductive cycles, systematics, and conservation of Philippine sea snakes. National Geographic Society Research Reports 1977. Vol. 18:343–349.

Gorzula, S. 1984. Proposal for a photographic method for size estimates of crocodilians. Herpetological Review 15:38–39.

Gorzula, S. 1996. The trade in dendrobatid frogs from 1987 to 1993. Herpetological Review 27:116–123.

Gorzula, S., W. O. Nsiah, and W. Odura. 1997. Survey of the status and management of the Royal Python (*Python regius*) in Ghana. Report to the Secretariat of the Convention of International Trade in Endangered Species of Wild Fauna and Flora (CITES). Geneva, Switzerland.

Gorzula, S., and A. E. Seijas. 1989. The common caiman. Pp. 44–61. In *Crocodiles: Their Ecology, Management, and Conservation.* Special Publication of the Crocodile Specialist Group of the Species Survival Commission of the International Union for Conservation of Nature and Natural Resources. Gland, Switzerland.

Gragg, J. E., G. H. Rodda, J. A. Savidge, G. C. White, K. Dean-Bradley, and A. R. Ellingson. 2007. Response of Brown Treesnakes to reduction of their rodent prey. Journal of Wildlife Management 71:2311–2317.

Graham, A., and R. Bell. 1969. Factors influencing the countability of animals. East African Agricultural and Forestry Journal 34 (Special Issue):38–43.

Graham, A. D. 1977. A management plan for Okavango crocodiles. Pp. 223–234. In Botswana Society, *Proceedings of the Symposium on the Okavango Delta and its Future Utilisation, National Museum, Gaborone, Botswana, August 30th to September 2nd, 1976.* Botswana Notes and Records, Special ed. 2.

Graham, T. E. 1979. Life history techniques. Pp. 73–95. In M. Harless and H. Morlock (eds.), *Turtles: Perspectives and Research.* Wiley, New York.

Graham, T. E. 1986. A warning against the use of Petersen disc tags in turtle studies. Herpetological Review 17:42–43.

Graham, T. E. 1995. Habitat use and population parameters of the Spotted Turtle, *Clemmys guttata*, a species of special concern in Massachusetts. Chelonian Conservation and Biology 1:207–214.

Graham, T. E., and A. A. Graham. 1992. Metabolism and behavior of wintering Common Map Turtles, *Graptemys geographica,* in Vermont. Canadian Field-Naturalist 106:517–519.

Graham, T. E., and A. A. Graham. 1997. Ecology of the Eastern Spiny Softshell, *Apalone spinifera spinifera,* in the Lamoille River, Vermont. Chelonian Conservation and Biology 2:363–369.

Grant, B. W., and A. E. Dunham. 1988. Thermally imposed time constraints on the activity of the desert lizard *Sceloporus merriami.* Ecology 69:167–176.

Grant, B. W., A. D. Tucker, J. E. Lovich, A. M. Mills, P. M. Dixon, and J. W. Gibbons. 1992. The use of coverboards in estimating patterns of reptile and amphibian biodiversity. Pp. 379–403. In D. R. McCullough and R. H. Barrett (eds.), *Wildlife 2001: Populations.* Elsevier Science, New York.

Grant, G. S., H. Malpass, and J. Beasley. 1996. Leatherback Turtle and jellyfish surveys on Topsail Island, North Carolina. Pp. 112–115. In J. A. Keinath, D. E. Barnard, J. A. Musick, and B. A. Bell (compilers), *Proceedings of the Fifteenth Annual Workshop on Sea Turtle Biology and Conservation.* NOAA Technical Memorandum NMFS-SEFSC-387. National Marine Fisheries Service, Miami, Florida.

Graves, B. M., and D. Duvall. 1987. An experimental study of aggregation and thermoregulation in Prairie Rattlesnakes, *Crotalus viridis viridis.* Herpetologica 43:259–264.

Graves, B. M., and D. Duvall. 1990. Spring emergence patterns of Wandering Garter Snakes and Prairie Rattlesnakes in Wyoming. Journal of Herpetology 24:351–356.

Gray, J. S. 1987. Species-abundance patterns. Pp. 53–67. In J. H. R. Gee and P. S. Giller (eds.), *Organization of Communities: Past and Present. 27th Symposium of the British Ecological Society, Aberystwyth, 1986.* Blackwell Scientific, Cambridge, Massachusetts.

Grayson, K. L., and M. E. Dorcas. 2004. Seasonal temperature variation in the Painted Turtle (*Chrysemys picta*). Herpetologica 60:325–336.

Green, A. J. 2001. Mass/length residuals: Measures of body condition or generators of spurious results? Ecology 82:1473–1483.

Green, B., and D. King. 1993. *Goanna: The Biology of Varanid Lizards*. New South Wales University Press, Kensington, New South Wales, Australia.

Greenbaum, E. 2002. A standardized series of embryonic stages for the emydid turtle *Trachemys scripta*. Canadian Journal of Zoology 80:1350–1370.

Greenbaum, E., and J. L. Carr. 2002. Staging criteria for embryos of the Spiny Softshell Turtle, *Apalone spinifera* (Testudines: Trionychidae). Journal of Morphology 254:272–291.

Greenberg, C. H., D. G. Neary, and L. D. Harris. 1994. A comparison of herpetofaunal sampling effectiveness of pitfall, single-ended, and double-ended funnel traps used with drift fences. Journal of Herpetology 28:319–324.

Greene, B. D., J. R. Dixon, J. M. Mueller, M. J. Whiting, and O. W. Thornton, Jr. 1994. Feeding ecology of the Concho Water Snake, *Nerodia harteri paucimaculata*. Journal of Herpetology 28:165–172.

Greene, B. D., J. R. Dixon, M. J. Whiting, and J. M. Mueller. 1999. Reproductive ecology of the Concho Water Snake, *Nerodia harteri paucimaculata*. Copeia 1999:701–709.

Greene, H. W. 1995. Nonavian reptiles as laboratory animals. ILAR Journal 37:182–186.

Greene, H. W. 1997. *Snakes: The Evolution of Mystery in Nature*. University of California Press, Berkeley, California.

Greer, A. E. 1970. Evolutionary and systematic significance of crocodilian nesting habits. Nature 227:523–524.

Greer, A. E. 1989. *The Biology and Evolution of Australian Lizards*. Surrey Beatty, Chipping Norton, New South Wales, Australia.

Gregory, P. T. 1974. Patterns of spring emergence of the Red-Sided Garter Snake (*Thamnophis sirtalis parietalis*) in the Interlake region of Manitoba. Canadian Journal of Zoology 52:1063–1069.

Gregory, P. T. 1983. Identification of sex of small snakes in the field. Herpetological Review 14:42–43.

Gregory, P. T. 1984a. Communal denning in snakes. Pp. 57–75. In R. A. Seigel, L. E. Hunt, J. L. Knight, L. Malaret, and N. L. Zuschlag (eds.), *Vertebrate Ecology and Systematics: A Tribute to Henry S. Fitch*. University of Kansas Publications, Museum of Natural History no. 10.

Gregory, P. T. 1984b. Habitat, diet, and composition of assemblages of Garter Snakes (*Thamnophis*) at eight sites on Vancouver Island. Canadian Journal of Zoology 62:2013–2022.

Gregory, P. T., G. J. Davies, and J. M. MacCartney. 1989. A portable device for restraining rattlesnakes in the field. Herpetological Review 20:43–44.

Gregory, P. T., and K. W. Larsen. 1993. Geographic variation in reproductive characteristics among Canadian populations of the Common Garter Snake (*Thamnophis sirtalis*). Copeia 1993: 946–958.

Grenard, S. 1991. *Handbook of Alligators and Crocodiles*. Krieger, Malabar, Florida.

Grenot C. J., L. Garcin, J. Dao, J.-P. Herold, B. Fahys, H. Tséré-Pagès. 2000. How does the European common lizard, *Lacerta vivipara,* survive the cold of winter? Comparative Biochemistry and Physiology A 127:71–80.

Greshock, J. D. 1998. Effects of Reproductive Status on the Habitat Use and Activity Patterns of the Northern Water Snake, *Nerodia sipedon sipedon*. Unpubl. Master's thesis, Villanova University, Villanova, Pennsylvania.

Griffiths, M., and C. P. van Schaik. 1993. The impact of human traffic on the abundance and activity periods of Sumatran rain forest wildlife. Conservation Biology 7:623–626.

Grigg, G. C., and F. Seebacher. 2001. Crocodilian thermal relations. Pp. 297–309. In G. C. Grigg, F. Seebacher, and C. E. Franklin (eds.), *Crocodilian Biology and Evolution*. Surrey Beatty, Chipping Norton, New South Wales, Australia.

Grigg, G. C., F. Seebacher, and C. E. Franklin. 2001. *Crocodilian Biology and Evolution*. Surrey Beatty, Chipping Norton, New South Wales, Australia.

Grismer, L. L. 1994. Three new species of intertidal side-blotched lizards (Genus *Uta*) from the Gulf of California, México. Herpetologica 50:451–474.

Grismer, L. L. 2002. *Amphibians and Reptiles of Baja California including its Pacific Islands and the Islands in the Sea of Cortés*. University of California Press, Berkeley, California.

Groombridge, B. 1987. The distribution and status of world crocodilians. Pp. 9–21. In G. J. W. Webb, S. C. Manolis, and P. J. Whitehead (eds.), *Wildlife Management: Crocodiles and Alligators*. Surrey Beatty and the Conservation Commission of The Northern Territory, Chipping Norton, New South Wales, Australia.

Grothe, S. 1992. Red-tailed Hawk Predation on Snakes: The Effects of Weather and Snake Activity. Unpubl. Master's thesis. Idaho State University, Pocatello, Idaho.

Gruber, B. 2004. Measuring activity of geckos with an automatic movement monitoring system. Herpetological Review 35:245–247.

Guilfoyle, M. P. 2010. *Implementing Herpetofaunal Inventory and Monitoring Efforts on Corps of Engineers Project Lands*. Final Report, ERDC/EL TR-10-5, U.S. Army Corps of Engineers, Washington, DC.

Guillette, L. J., Jr., D. A. Crain, M. P. Gunderson, S. A. E. Kools, M. R. Milnes, E. F. Orlando, A. A. Rooney, and A. R. Woodward. 2000. Alligators and endocrine disrupting contaminants: A current perspective. American Zoologist 40:438–452.

Guinea, M. L. 1994. Sea snakes of Fiji and Niue. Pp. 212–233. In P. Gopalakrishnakone (ed.), *Sea Snake Toxicology*. Singapore University Press, Singapore.

Guinea, M. L. 1996. Functions of the cephalic scales of the sea snake *Emydocephalus annulatus*. Journal of Herpetology 30:126–128.

Gunning, G. E., and W. M. Lewis. 1957. An electrical shocker for the collection of amphibians and reptiles in the aquatic environment. Copeia 1957:52.

Guyer, C. 1990. The herpetofauna of La Selva, Costa Rica. Pp. 371–385. In A. H. Gentry (ed.), *Four Neotropical Rainforests*. Yale University Press, New Haven, Connecticut.

Guyer, C., M. A. Bailey, J. Holmes, J. Stiles, and S. Stiles. 2007. *Herpetofaunal Response to Longleaf Pine Ecosystem Restoration, Conecuh National Forest, Alabama*. Unpubl. report to the US Forest Service, Auburn University, Auburn, Alabama. http://www.outdooralabama.com/ [click on: research/management> state wildlife grants> projects funded by SWG]

Guyer, C., and M. A. Donnelly. 2005. *Amphibians and Reptiles of La Selva, Costa Rica, and the Caribbean Slope: A Comprehensive Guide*. University of California Press, Berkeley, California.

Guyer, C., C. T. Meadows, S. C. Townsend, and L. G. Wilson. 1997. A camera device for recording vertebrate activity. Herpetological Review 28:138–140.

Gyi, K. K. 1970. A revision of colubrid snakes of the subfamily Homalopsinae. University of Kansas Publications, Museum of Natural History 20:47–223.

Hain, J. H. W. 1992. *Airships for Marine Mammal Research: Evaluation and Recommendations*. Report number T-68108863 Marine Mammal Commission, Washington, DC. [Access no. PB92-128271, National Technical Information Service, Springfield, Virginia.]

Hain, J. H. W., S. L. Ellis, R. D. Kenney, and C. K. Slay. 1999. Sightability of right whales in coastal waters of the southeastern United States with implications for the aerial monitoring program. Pp. 191–207. In G. W. Garner, S. C. Amstrup, J. L. Laake, B. F. J. Manly, L. L. McDonald, and D. G. Robertson (eds.), *Marine Mammal Survey and Assessment Methods*. Balkema, Rotterdam, The Netherlands.

Hall, E. R. 1981. *The Mammals of North America*. Vols. 1 and 2. 2nd ed. Wiley, New York.

Hallé, F., and C. Blanc (eds.). 1990. *Biologie d'une Canopée de Forêt Equatoriale: Rapport de Mission Radeau des Cimes, Octobre–Novembre 1989, Petie-Saut, Guyane Française*. Association [[AQ22]]OPRDC, Montpellier, France.

Halliday, T. R., and P. A. Verrell. 1988. Body size and age in amphibians and reptiles. Journal of Herpetology 22:253–265.

Hamilton, J. D. 1994. *Time Series Analysis*. Princeton University Press, Princeton, New Jersey.

Hammer, D. A. 1969. Parameters of a marsh Snapping Turtle population, La Creek Refuge, South Dakota. Journal of Wildlife Management 33:995–1005.

Hampton, P. 2007. A comparison of the success of artificial cover types for capturing amphibians and reptiles. Amphibia-Reptilia 28:433–437.

Hampton, P. M., and N. E. Haertle. 2009. A new view from a novel squeeze box design. Herpetological Review 40:44.

Han, K. H., R. B. Steubing, and H. K. Voris. 1991. Population structure and reproduction in the marine snake *Lapemis hardwickii* Gray, from the west coast of Sabah. Sarawak Museum Journal 42(63):463–475.

Hanley, K. A., D. M. Vollmer, and T. J. Case. 1995. The distribution and prevalence of helminths, coccidia and blood parasites in two competing species of gecko: Implications for apparent competition. Oecologia 102:220–229.

Harden, L. A., N. A. DiLuzio, J. W. Gibbons, and M. E. Dorcas. 2007. Spatial and thermal ecology of Diamondback Terrapins (*Malaclemys terrapin*) in a South Carolina salt marsh. Journal of the North Carolina Academy of Science 123:154–162.

Harden, L. A., and M. E. Dorcas. 2008. Using thermal biology to investigate habitat use and activity of mud turtles (*Kinosternon subrubrum*) on a golf course. Pp. 321–323. In J. C. Mitchell, R. E. Jung Brown, and B. Bartholomew (eds.), *Urban Herpetology.* Herpetological Conservation Vol. 3. Society for the Study of Amphibians and Reptiles. Salt Lake City, Utah.

Harding, K. A. 1977. The use of ketamine anaesthesia to milk two tropical rattlesnakes (*Crotalus durissus terrificus*). Veterinary Record 100:289–290.

Hardy, D. L., Sr., and H. W. Greene. 1999. Surgery on rattlesnakes in the field for implantation of transmitters. Sonoran Herpetologist 12:26–28.

Hardy, D. L., Sr., and H. W. Greene. 2000. Inhalation anesthesia of rattlesnakes in the field for processing and transmitter implantation. Sonoran Herpetologist 13:110–114.

Harlow, P. S. 1996. A harmless technique for sexing hatchling lizards. Herpetological Review 27:71–72.

Harlow, P. S., and P. N. Biciloa. 2001. Abundance of the Fijian Crested Iguana (*Brachylophus vitiensis*) on two islands. Biological Conservation 98:223–231.

Harlow, P., and R. Shine. 1988. Voluntary ingestion of radiotransmitters by snakes in the field. Herpetological Review 19:57.

Harper, S. J., and G. O. Batzli. 1996. Monitoring use of runways by voles with passive integrated transponders. Journal of Mammalogy 77:364–369.

Harris, D. J. 2003. Can you bank on GenBank? Trends in Ecology and Evolution 18:317–319.

Harris, D. R. 1965. A technique for collecting aquatic reptiles and amphibians. Journal of the Ohio Herpetological Society 5:35–36.

Harvey, D. S. 2005. Detectability of a large-bodied snake (*Sistrurus c. catenatus*) by time-constrained searching. Herpetological Review 36:413–415.

Hasumi, M., and Y. G. Watanabe. 2007. An efficient method for skeletochronology. Herpetological Review 38:404–406.

Hatcher, R. T., and J. H. Shaw. 1981. A comparison of three indices to furbearer populations. Wildlife Society Bulletin 9:153–156.

Hathaway, S., R. N. Fisher, C. Rochester, C. Haas, M. Mendelsohn, G. Turschak, D. Stokes, M. Madden-Smith, E. Ervin, K. Pease, and C. Brown. 2004. *Baseline Biodiversity Survey for the Santa Ysabel Ranch Open Space Preserve*. USGS Western Ecological Research Center Technical Report. Prepared for: The Nature Conservancy and San Diego County Department of Parks and Recreation. http://www.werc.usgs.gov/ProductsSearch.aspx

Hathaway, S., J. O'Leary, R. Fisher, C. Rochester, C. Brehme, C. Hass, S. McMillan, M. Mendelsohn, D. Stokes, K. Pease, C. Brown, B. Yang, E. Ervin, M. Warburton, and M. Madden-Smith. 2002. *Baseline Biodiversity Survey for the Rancho Jamul Ecological Reserve*. USGS Western Ecological Research Center Final Report. Prepared for California Department of Fish and Game, Sacramento, California.

Hayat, M. A. 2000. *Principles and Techniques of Electron Microscopy: Biological Applications.* 4th ed. Cambridge University Press, New York.

Hayek, L. C. 1994a. Research design for quantitative amphibian studies. Pp. 21–39. In W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster (eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Smithsonian Institution Press, Washington, DC.

Hayek, L. C. 1994b. Analysis of amphibian biodiversity data. Pp. 207–269. In W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster (eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Smithsonian Institution Press, Washington, DC.

Hayek, L. C., and M. A. Buzas. 2010. *Surveying Natural Populations*: Quantitative Tools for Assessing Biodiversity. 2nd ed. Columbia University Press, New York.

Hayek, L. C., and R. Heyer. 2003. Adaptive cluster sampling for amphibians. Froglog 55:2–4. [Newsletter of the Declining Amphibian Populations Task Force, http://www.open.ac.uk/daptf/froglog/FROGLOG-55-4.html]

Hayek, L. C., and W. R. Heyer. 2005. Determining sexual dimorphism in frog measurement data: Integration of statistical significance, measurement error, effect size and biological significance. Anais da Academia Brasileira de Ciências 77:45–76.

Hayek, L. C., and R. W. McDiarmid. 1994. GIS and remote sensing techniques. Pp. 166–171. In W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster (eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians.* Smithsonian Institution Press, Washington, DC.

Hayes, W. K., K. R. Beaman, M. D. Cardwell, and S. P. Bush (eds.). 2008. *The Biology of Rattlesnakes.* Loma Linda University Press, Loma Linda, California.

Hayes-Odum, L. A., and D. Jones. 1993. Effects of drought on American Alligators (*Alligator mississippiensis*) in Texas. Texas Journal of Science 45:182–185.

He, F., and D. Tang. 2008. Estimating the niche preemption parameter of the geometric series. Acta Oecologica 33:105–107.

Heatwole, H. 1975. Sea snakes found on reefs in the southern Coral Sea (Saumarez, Swains, Cato Island). Pp. 163–171. In W. A. Dunson (ed.), *The Biology of Sea Snakes*. University Park Press, Baltimore, Maryland.

Heatwole, H. 1982. A review of structuring in herpetofaunal assemblages. Pp. 1–19. In N. J. Scott, Jr. (ed.), *Herpetological Communities*. U.S. Department of the Interior, Fish and Wildlife Service, Wildlife Research Report 13.

Heatwole, H. 1989. The concept of the econe, a fundamental ecological unit. Tropical Ecology 30:13–19.

Heatwole, H. 1999. *Sea Snakes*. Krieger, Malabar, Florida.

Heatwole, H., S. Busack, and H. Cogger. 2005. Geographic variation in sea kraits of the *Laticauda colubrina* complex (Serpentes: Elapidae: Hydrophiinae: Laticaudini). Herpetological Monographs 19:1–136.

Heatwole, H., and O. J. Sexton. 1966. Herpetofaunal comparisons between two climatic zones in Panama. American Midland Naturalist 75:45–60.

Heatwole, H., and B. L. Stuart. 2008. High densities of a "rare skink." Herpetological Review 39:169–170.

Hebrard, J. J., and R. C. Lee. 1981. A large collection of brackish water snakes from the central Atlantic coast of Florida. Copeia 1981:886–889.

Heck, K. L., Jr., G. Van Belle, and D. Simberloff. 1975. Explicit calculation of the rarefaction diversity measurement and the determination of sufficient sample size. Ecology 56:1459–1461.

Hedges, S. B., A. Couloux, and N. Vidal. 2009. Molecular phylogeny, classification, and biogeography of West Indian racer snakes of the tribe Alsophiini (Squamata, Dipsadidae, Xenodontinae). Zootaxa 2067:1–28.

Hedges, S. B., and R. Thomas. 1991. The importance of systematic research in the conservation of amphibian and reptile populations. Pp. 56–61. In J. A. Moreno (ed.), *Status y Distribución de los Reptiles y Anfíbios de la Región de Puerto Rico.* Departamento de Recursos Naturales de Puerto Rico, Publicación Científica Miscelánea no. 1.

Hein, E. W., and W. F. Andelt. 1994. Evaluation of coyote attractants and an oral delivery device for chemical agents. Wildlife Society Bulletin 22:651–655.

Hellman, R. E., and S. R. Telford. 1956. Notes on a large number of Red-Bellied Mudsnakes, *Farancia a. abacura,* from northcentral Florida. Copeia 1956:257–258.

Henderson, R. W. 1974. Aspects of the ecology of the juvenile Common Iguana (*Iguana iguana*). Herpetologica 30:327–332.

Henderson, R. W., and R. Powell (eds.). 2007. *Biology of the Boas and Pythons.* Eagle Mountain Publishing, Eagle Mountain, Utah.

Henderson, R. W., and R. Powell. 2009. *Natural History of West Indian Reptiles and Amphibians*. University Press of Florida, Gainesville, Florida.

Henderson, R. W., and R. A. Winstel. 1995. Aspects of habitat selection by an arboreal boa (*Corallus enydris*) in an area of mixed agriculture on Grenada. Journal of Herpetology 29:272–275.

Hendrie, D. B. 2000. Status and conservation of tortoises and freshwater turtles in Vietnam. Pp. 63–73. In P. P. van Dijk, B. L. Stuart, and A. G. J. Rhodin (eds.), *Asian Turtle Trade: Proceedings of a Workshop on Conservation and Trade of Freshwater Turtles and Tortoises in Asia, Phnom Penh, Cambodia, 1–4 December 1999.* Chelonian Research Monographs 2.

Henke, S. E. 1998. The effect of multiple search items and item abundance on the efficiency of human searchers. Journal of Herpetology 32:112–115.

Henley, G. B. 1981. A new technique for recognition of snakes. Herpetological Review 12:56.

Hensley, M. M. 1950. Results of a herpetological reconnaissance in extreme southwestern Arizona and adjacent Sonora, with a description of a new subspecies of the Sonoran Whipsnake (*Masticophis bilineatus*). Transactions Kansas Academy of Science 53:270–288.

Henwood, T., and S. P. Epperly. 1999. Aerial surveys in foraging habitats. Pp. 65–66. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, and M. Donnelly (eds.), *Research and Management Techniques for the Conservation of Sea Turtles*. IUCN/SSC Marine Turtle Specialist Group Publication no. 4.

Heppell, S. 2000. North Carolina aerial transect surveys 1988–1991. Pp. 64–65. In K. A. Bjorndal and A. B. Bolten (eds.), *Proceedings of a Workshop on Assessing Abundance and Trends for In-Water Sea Turtle Populations*. NOAA Technical Memorandum NMFS-SEFSC-445. National Marine Fisheries Service, Miami, Florida.

Hermann, S. M., C. Guyer, J. H. Waddle, and M. G. Nelms. 2002. Sampling on private property to evaluate population status and effects of land use practices on the Gopher Tortoise, *Gopherus polyphemus*. Biological Conservation 108:289–298.

Herzog, H. A., and S. Galvin. 1997. Common sense and the mental lives of animals: An empirical approach. Pp. 237–253. In R. W. Mitchell, N. S. Thompson, and H. L. Miles (eds.), *Anthropomorphism, Anecdotes, and Animals*. State University of New York Press, Albany, New York.

Heske, E. J. 1995. Mammalian abundances on forest-farm edges versus forest interiors in southern Illinois: Is there an edge effect? Journal of Mammalogy 76:562–568.

Heyer, W. R., M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster (eds.). 1994a. *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Smithsonian Institution Press, Washington, DC.

Heyer, W. R., M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster. 1994b. Essentials of standardization and quantification. Pp. 17–20. In W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster (eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Smithsonian Institution Press, Washington, DC.

Heywood, D. I., S. Cornelius, and S. Carver. 1998. *An Introduction to Geographical Information Systems*. Prentice Hall, Upper Saddle River, New Jersey.

Hiaasen, C. 1991. *Native Tongue: A Novel*. Knopf, New York.

Hiby, L. 1999. The objective identification of duplicate sightings in aerial survey for porpoise. Pp. 179–189. In G. W. Garner, S. C. Amstrup, J. L. Laake, B. F. J. Manly, L. L. McDonald, and D. G. Robertson (eds.), *Marine Mammal Survey and Assessment Methods*. Balkema, Rotterdam, The Netherlands.

Hiby, L., and P. Lovell. 1998. Using aircraft in tandem formation to estimate abundance of Harbour Porpoise. Biometrics 54:1280–1289.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25:1965–1978.

Hildebrand, H. H. 1982. A historical review of the status of sea turtle populations in the western Gulf of Mexico. Pp.447–453. In K. A. Bjorndal (ed.), *Biology and Conservation of Sea Turtles*. Smithsonian Institution Press and World Wildlife Fund, Washington, DC.

Hildebrand, M. 1968. *Anatomical Preparations*. University of California Press, Berkeley, California.

Hillis, D. M., C. Moritz, and B. K. Mable (eds.). 1996. *Molecular Systematics*. 2nd ed. Sinauer, Sunderland, Massachusetts.

Hines, J. E. 2006. PRESENCE2-Software to estimate patch occupancy and related parameters. U.S. Geological Survey-Patuxent Wildlife Research Center. [See http://www.mbr-pwrc.usgs.gov/software/presence.html for updated versions.]

Hines, T. C., and C. L. Abercrombie III. 1987. The management of alligators in Florida, USA. Pp. 43–47. In G. J. W. Webb, S. C. Manolis, and P. J. Whitehead (eds.), *Wildlife Management: Crocodiles and Alligators*. The Conservation Commission of the Northern Territory and Surrey Beatty, Chipping Norton, New South Wales, Australia.

Hinton, T. G., P. D. Fledderman, J. E. Lovich, J. D. Congdon, and J. W. Gibbons. 1997. Radiographic determination of fecundity: Is the technique safe for developing turtle embryos? Chelonian Conservation and Biology 2:409–414.

Hirth, H. F. 1966. The ability of two species of snakes to return to a hibernaculum after displacement. Southwestern Naturalist 11:49–53.

Hobbs, T. J., S. R. Morton, P. Masters, and K. R. Jones. 1994. Influence of pit-trap design on sampling of reptiles in arid spinifex grasslands. Wildlife Research 21:483–490.

Hodson, L. A., and J. F. W. Pearson. 1943. Notes on the discovery and biology of two Bahaman fresh-water turtles of the genus *Pseudemys*. *Proceedings of the Florida Academy of Sciences* 6(2):17–23.

Hofer, U., and L.-F. Bersier. 2001. Herpetofaunal diversity and abundance in tropical upland forests of Cameroon and Panama. Biotropica 33:142–152.

Hoffman, M., et al. [174 co-authors]. 2010. The impact of conservation on the status of the world's vertebrates. Science: published online 26 October 2010; 10.1126/science.1194442

Holdridge, L. R., W. C. Grenke, W. H. Hatheway, T. Liang, and J. A. Tosi. 1971. *Forest Environments in Tropical Life Zones: A Pilot Study*. Pergamon, Oxford, England

Holland, D. C. 1994. *The Western Pond Turtle: Habitat and History*. Final Report DOE/BP 62137-1. Prepared for: U.S. Department of Energy, Bonneville Power Administration, Division of Environment, Fish and Wildlife, Portland, Oregon. http://www.efw.bpa.gov/searchpublications/#

Hollands, M. 1987. The management of crocodiles in Papua New Guinea. Pp. 73–89. In G. J. W. Webb, S. C. Manolis, and P. J. Whitehead (eds.), *Wildlife Management: Crocodiles and Alligators*. Surrey Beatty, Chipping Norton, New South Wales, Australia.

Holling, C. S. (ed.). 1978. *Adaptive Environmental Assessment and Management*. Wiley, New York.

Holtzman, D. A., and M. Halpern. 1989. In vitro technique for studying garter snake (*Thamnophis* sp.) development. Journal of Experimental Zoology 250:283–288.

Holycross, A. T. 1995. *Crotalus viridis* (Western Rattlesnake). Phenology. Herpetological Review 26:37–38.

Hooge, P. N., and B. Eichenlaub. 1997. *Animal Movement Extension to ArcView. Ver. 1.1.* Alaska Science Center - Biological Science Office, U.S. Geological Survey, Anchorage, Alaska.

Hoogmoed, M., and T. C. S. Avila-Pires. 1990. Observations on the herpetofauna of Petit Saut, Sinnamary River, French Guyana. Pp. 222–229. In F. Hallé, and P. Blanc (eds.), *Biologie d'une Canopée de Forêt Equatoriale: Rapport de Mission Radeau des Cimes, Octobre–Novembre 1989, Petie-Saut, Guyane Française*. Association OPRDC Montpellier, France.

Hoogmoed, M. S., and T. C. S. Avila-Pires. 1991. Annotated checklist of the herpetofauna of Petit Saut, Sinnamary River, French Guyana. Zoologische Mededelingen Leiden 65:53–88.

Hopkins-Murphy, S. R., and T. M. Murphy. 1988. Status of the Loggerhead Turtle in South Carolina. Pp. 35–38. In B. A. Schroeder (compiler), *Proceedings of the Eighth Annual Conference on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFC-214. National Marine Fisheries Service, Miami, Florida.

Hopkins-Murphy, S. R., and T. M. Murphy. 1994. Status of the loggerhead nesting population in South Carolina: A five year up-date. Pp. 62–64. In K. A. Bjorndal, A. B. Bolten, D. A. Johnson, and P. J. Eliazar (compilers), *Proceedings of the Fourteenth Annual Symposium on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFSC-351. National Marine Fisheries Service, Miami, Florida.

Horner, P. 1992. *Skinks of the Northern Territory*. Northern Territory Museum of Arts and Sciences, Handbook no. 2. Darwin, Australia.

Hoser, R. 2008. A technique for artificial insemination in squamates. Bulletin of the Chicago Herpetological Society 43:1–9.

Hosmer, D. W., and S. Lemeshow. 2000. *Applied Logistic Regression*. 2nd ed. John Wiley, New York.

Houston, D., and R. Shine. 1994a. Movements and activity patterns of Arafura Filesnakes (Serpentes: Acrochordidae) in tropical Australia. Herpetologica 50:349–357.

Houston, D., and R. Shine. 1994b. Low growth rates and delayed maturation in Arafura Filesnakes (Serpentes: Acrochordidae) in tropical Australia. Copeia 1994:726–731.

Houze, C. M., Jr., and C. R. Chandler. 2002. Evaluation of coverboards for sampling terrestrial salamanders in South Georgia. Journal of Herpetology 36:75–81.

Hoyer, R. F., and G. R. Stewart. 2000. Biology of the Rubber Boa (*Charina bottae*), with emphasis on *C. b. umbratica*. Part I. Capture, size, sexual dimorphism, and reproduction. Journal of Herpetology 34:348–354.

Hoyle, M. E., and J. W. Gibbons. 2000. Use of a marked population of Diamondback Terrapins (*Malaclemys terrapin*) to determine impacts of recreational crab pots. Chelonian Conservation and Biology 3:735–737.

Hua, T.-m., C.-l. Wang, and B.-h. Chen. 2004. Stages of embryonic development for *Alligator sinensis*. Zoological Research 25:263–271. [in Chinese with English abstract]

Huber, J. T. 1998. The importance of voucher specimens, with practical guidelines for preserving specimens of the major invertebrate phyla for identification. Journal of Natural History 32:367–385.

Hubert, J., and J. P. Dufaure. 1968. Table de développement de la vipère aspic: *Vipera aspis* L. Bulletin de la Société Zoologique de France 93:135–148.

Hudnall, J. A. 1982. New methods for measuring and tagging snakes. Herpetological Review 13:97–98.

Hudson, S. 1996. Natural toe loss in southeastern Australian skinks: Implications for marking lizards by toe-clipping. Journal of Herpetology 30:106–110.

Huey, R. B. 1982. Temperature, physiology, and the ecology of reptiles. Pp. 25–91. In C. Gans and F. H. Pough (eds.), *Biology of the Reptilia,* Vol. 12. *Physiology C. Physiological Ecology.* Academic Press, New York.

Huey, R. B., A. E. Dunham, K. L. Overall, and R. A. Newman. 1990. Variation in locomotor performance in demographically known populations of the lizard *Sceloporus merriami*. Physiological Zoology 63:845–872.

Huey, R. B., C. R. Peterson, S. J. Arnold, and W. P. Porter. 1989. Hot rocks and not-so-hot rocks: Retreat-site selection by garter snakes and its thermal consequences. Ecology 70: 931–944.

Hugall, A. F., R. Foster, and M. S. Y. Lee. 2007. Calibration choice, rate smoothing, and the pattern of tetrapod diversification according to the long nuclear gene RAG-1. Systematic Biology 56:543–563.

Huheey, J. E. 1963. Concerning the use of paraformaldehyde as a field preservative. Copeia 1963:192–193.

Hulse, A. C. 1982. Reproduction and population structure in the turtle, *Kinosternon sonoriense*. Southwestern Naturalist 27:447–456.

Hurlbert, S. H. 1971. The nonconcept of species diversity: A critique and alternative parameters. Ecology 52:577–586.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54:187–211.

Hutton, J. M. 1986. Age determination of living Nile Crocodiles from the cortical stratification of bone. Copeia 1986:332–341.

Hutton, J. M. 1987. Techniques for aging wild crocodilians. Pp. 211–216. In G. J. W. Webb, S. C. Manolis, and P. J. Whitehead (eds.), *Wildlife Management: Crocodiles and Alligators*. Surrey Beatty, Chipping Norton, New South Wales, Australia.

Hutton, J. M., J. P. Loveridge, and D. K. Blake. 1987. Capture methods for the Nile crocodile in Zimbabwe. Pp. 243–247. In G. J. W. Webb, S. C. Manolis, and P. J. Whitehead (eds.), *Wildlife Management: Crocodiles and Alligators*. Surrey Beatty, Chipping Norton, New South Wales, Australia.

Hyde, E. J., and T. R. Simons. 2001. Sampling plethodontid salamanders: Sources of variability. Journal of Wildlife Management 65:624–632.

Hyslop, N. L., J. M. Meyers, R. J. Cooper, and T. M. Norton. 2009. Survival of radio-implanted *Drymarchon couperi* (Eastern Indigo Snake) in relation to body size and sex. Herpetologica 65:199–206.

Imler, R. H. 1945. Bullsnakes and their control on a Nebraska wildlife refuge. Journal of Wildlife Management 9:265–273.

Ineich, I. 2003. Contribution à la connaissance de la biodiversité des régions afro-montagnardes: Les reptiles du Mont Nimba. Pp. 597–637. In M. LaMotte and R. Roy (eds.), Le Peuplement animal du Mont Nimba (Guinée, Côte d'Ivoire, Liberia). Mémoires du Muséum National d'Histoire Naturelle 190.

Ineich, I., and P. LaBoute. 2002. *Sea Snakes of New Caledonia*. IRD Éditions, Paris, France.

Inger, R. F. 1980. Densities of floor-dwelling frogs and lizards in lowland forests of Southeast Asia and Central America. American Naturalist 115:761–770.

Inger, R. F. 1983. Morphological and ecological variation in the flying lizards (genus *Draco*). Fieldiana Zoology n. s. 18:1–35.

Inger, R. F., and R. K. Colwell. 1977. Organization of contiguous communities of amphibians and reptiles in Thailand. Ecological Monographs 47:229–253.

Ingram, G. J. 1979. The occurrence of lizards of the genus *Emoia* (Lacertilia, Scincidae) in Australia. Memoirs of the Queensland Museum 19:431–437.

International Air Transport Association [IATA]. 2010. Dangerous Goods Regulations (DGR). 51st ed. International Air Transport Association, Montreal, Quebec, Canada.

Ireland, T. T., G. L. Wolters, and S. D. Schemnitz. 1994. Recolonization of wildlife on a coal strip-mine in northwestern New Mexico. Southwestern Naturalist 39:53–57.

Isaaks, E. H., and R. M. Srivastava. 1989. *An Introduction to Applied Geostatistics*. Oxford University Press, New York.

ITU (International Telecommunication Union). The World in 2010: ICT Facts and Figures. http://www.itu.int/ITU-D/ict/material/FactsFigures2010.pdf [accessed November 2010].

Iungman, J., C. I. Piña, and P. Siroski. 2008. Embryological development of *Caiman latirostris* (*Crocodylia: Alligatoridae*). Genesis 46:401–417.

Iverson, J. B. 1977. Geographic variation in the musk turtle, *Sternotherus minor.* Copeia 1977:502–517.

Iverson, J. B. 1979a. Another inexpensive turtle trap. Herpetological Review 10:55.

Iverson, J. B. 1979b. The female reproductive cycle in north Florida *Kinosternon baurii* (Testudines: Kinosternidae). Brimleyana 1:37–46.

Iverson, J. B. 1979c. Behavior and ecology of the rock iguana, *Cyclura carinata*. Bulletin of the Florida State Museum, Biological Sciences 24:175–358.

Iverson, J. B. 1989. Natural history of the Alamos Mud Turtle, *Kinosternon alamosae* (Kinosternidae). The Southwestern Naturalist 34:134–142.

Iverson, J. B. 1991. Life history and demography of the Yellow Mud Turtle, *Kinosternon flavescens*. Herpetologica 47:373–395.

Iverson, J. B. 1992. *A Revised Checklist with Distribution Maps of the Turtles of the World.* Privately Printed, Richmond, Indiana.

IWGSC (Interagency Working Group on Scientific Collections). 2009. *Scientific Collections: Mission Critical Infrastructure for Federal Science Agencies. A Report.* National Science and Technology Council, Washington, DC. http://www.whitehouse.gov/sites/default/files/sci-collections-report-2009-rev2.pdf

Jackson, C. G. 1969. Agonistic behavior in *Sternotherus minor minor* Agassiz. Herpetologica 25:53–54.

Jackson, K. 2002. Post-ovipositional development of the Monocled Cobra, *Naja kaouthia* (Serpentes: Elapidae). Zoology 105:203–214.

Jacob, J. S., and C. W. Painter. 1980. Overwinter thermal ecology of *Crotalus viridis* in the north-central plains of New Mexico. Copeia 1980:799–805.

Jacobs, L. (ed.). 1982. *Parasitic Zoonoses.* Vol. 1. Part 1. *Protozoan Zoonoses.* CRC Handbook Series in Zoonoses, Section C. CRC Press, Boca Raton, Florida.

Jacobson, E. R., J. Schumacher, and M. Green. 1992. Field and clinical techniques for sampling and handling blood for hematologic and selected biochemical determinations in the Desert Tortoise, *Xerobates agassizii*. Copeia 1992:237–241.

Jacobson, H. A., J. C. Kroll, R. W. Browning, B. H. Koerth, and M. H. Conway. 1997. Infrared-triggered cameras for censusing White-tailed Deer. Wildlife Society Bulletin 25:547–556.

Jaeger, R. G., and R. F. Inger. 1994. 4. Quadrat Sampling. Pp. 97–102. In W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster (eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Smithsonian Institution Press, Washington, DC.

James, C., and R. Shine. 1985. The seasonal timing of reproduction: A tropical-temperate comparison in Australian lizards. Oecologia (Berlin) 67:464–474.

James, F. C., and H. H. Shugart, Jr. 1970. A quantitative method of habitat description. Audubon Field Notes 24:727–736.

Jamniczky, H. A., and A. P. Russell. 2008. Carotid circulatory development in turtles: Using existing material to seek critical developmental stages that localize establishment of clade-specific patterns. Amphibia-Reptilia 29:270–277.

Janzen, F. J. 1994. Climate change and temperature-dependent sex determination in reptiles. *Proceedings of the National Academy of Sciences (USA)* 91:7487–7490.

Janzen, F. J., and G. Paukstis. 1991. Environmental sex determination in reptiles: Ecology, evolution, and experimental design. Quarterly Review of Biology 66:149–179.

Jayne, B. C., T. J. Ward, and H. K. Voris. 1995. Morphology, reproduction, and diet of the marine homalopsine snake *Bitia hydroides* in peninsular Malaysia. Copeia 1995:800–808.

Jeffery, J. E., O. R. P. Bininda-Emonds, M. I. Coates, and M. K. Richardson. 2002. Analyzing evolutionary patterns in amniote embryonic development. Evolution and Development 4:292–302.

Jemison, S. C., L. A. Bishop, P. G. May, and T. M. Farrell. 1995. The impact of PIT-tags on growth and movement of the rattlesnake, *Sistrurus miliarius*. Journal of Herpetology 29:129–132.

Jenkins, L. N., T. J. Thomasson IV, and J. G. Byrd. 2001. A field study of the Black Kingsnake *Lampropeltis getula nigra*. Herpetological Natural History 8:57–67.

Jenkins, R. W. G., and M. A. Forbes. 1985. Seasonal variation in abundance and distribution of *Crocodylus porosus* in the tidal East Alligator River, Northern Australia. Pp. 63–69. In G. Grigg, R. Shine, and H. Ehmann (eds.), *Biology of Australasian Frogs and Reptiles*. Royal Zoological Society of New South Wales and Surrey Beatty, Shippping Norton, New South Wales, Australia.

Jensen, J. R. 1996. *Introductory Digital Image Processing: A remote sensing perspective*. 2nd ed. Prentice Hall, Upper Saddle River, New Jersey.

Joanen, T. 1969. Nesting ecology of alligators in Louisiana. Pp. 141–151. In *Proceedings of the Twenty-third Annual Conference, Southeastern Association of Game and Fish Commissioners, 1969*.

Joanen, T., and L. McNease. 1970. A telemetric study of nesting female alligators on Rockefeller Refuge, Louisiana. Pp. 175–193. In *Proceedings of the Twenty-fourth Annual Conference, Southeastern Association of Game and Fish Commissioners, 1970*.

Joanen, T., and L. McNease. 1979. The cloacal sexing method for immature alligators. Pp. 179–181. In *Proceedings of the Thirty-second Annual Conference, Southeastern Association of Fish and Wildlife Agencies, 1978*.

Joanen, T., and L. McNease. 1987. The management of alligators in Louisiana, USA. Pp. 33–42. In G. J. W. Webb, S. C. Manolis, and P. J. Whitehead (eds.), *Wildlife Management: Crocodiles and Alligators*. Surrey Beatty, Chipping Norton, New South Wales, Australia.

Johnston, K., J. M. Ver Hoef, K. Krivoruchko, and N. Lucas. 2001. *Using ArcGIS Geostatistical Analyst*. ESRI, Redlands, California.

Jones, D., and L. Hayes-Odum. 1994. A method for the restraint and transport of crocodilians. Herpetological Review 25:14–15.

Jones, F. K., Jr. 1966. Techniques and methods used to capture and tag alligators in Florida. Pp. 98–101. In *Proceedings of the Nineteenth Annual Conference, Southeastern Association of Game and Fish Commissioners, 1965*.

Jones, J. C., and J. D. Reynolds. 1996. Environmental variables. Pp. 281–316. In W. J. Sutherland (ed.), *Ecological Census Techniques, A Handbook*. Cambridge University Press, Cambridge, England.

Jones, K. B. 1981. Effects of grazing on lizard abundance and diversity in western Arizona. Southwestern Naturalist 26:107–115.

Jones, K. B. 1986. Amphibians and reptiles. Pp. 267–290. In A. Y. Cooperrider, R. J. Boyd, and H. R. Stuart (eds.), *Inventory and Monitoring of Wildlife Habitat*. U.S. Department of the Interior, Bureau of Land Management Service Center, Denver, Colorado.

Jones, L. L. C., and R. E. Lovich (eds.). 2009. *Lizards of the American Southwest. A Photographic Field Guide*. Rio Nuevo, Tucson, Arizona.

Jones, S. M., and G. W. Ferguson. 1980. The effect of paint marking on mortality in a Texas population of *Sceloporus undulatus*. Copeia 1980:850–854.

Jones, T. R., R. D. Babb, F. R. Hensley, C. LiWanPo, and B. K. Sullivan. 2011. Sonoran Desert snake communities at two sites: Concordance and effects of increased road traffic. Herpetological Conservation and Biology 6:61–71.

Jorgensen, E. E., M. Vogel, and S. Demarais. 1998. A comparison of trap effectiveness for reptile sampling. Texas Journal of Science 50:235–242.

Judd, F. W. 1975. Activity and thermal ecology of the Keeled Earless Lizard, *Holbrookia propinqua*. Herpetologica 31:137–150.

Karanth, K. U., and J. D. Nichols. 1998. Estimation of tiger densities in India using photographic captures and recaptures. Ecology 79:2852–2862.

Karns, D. R. 1986. Field Herpetology. Methods for the Study of Amphibians and Reptiles in Minnesota. James Ford Bell Museum of Natural History, Occasional Paper No. 18.

Karns, D. R., H. K. Voris, and T. G. Goodwin. 2002. Ecology of Oriental-Australian rear-fanged water snakes (Colubridae: Homalopsinae) in the Pasir Ris Park Mangrove Forest, Singapore. Raffles Bulletin of Zoology 50:487–498.

Kauffeld, C. 1957. *Snakes and Snake Hunting*. Hanover House, Garden City, New York.

Kazmaier, R. T., E. C. Hellgren, D. C. Ruthven, III, and D. R. Synatzske. 2001. Effects of grazing on the demography and growth of the Texas Tortoise. Conservation Biology 15:1091–1101.

Kearney, M. 2002. Hot rocks and much-too-hot rocks: Seasonal patterns of retreat-site selection by a nocturnal ectotherm. Journal of Thermal Biology 27:205–218.

Keck, M. B. 1994a. A new technique for sampling semi-aquatic snake populations. Herpetological Natural History 2:101–103.

Keck, M. B. 1994b. Test for detrimental effects of pit tags in neonatal snakes. Copeia 1994:226–228.

Keinath, J. A., J. A. Musick, and R. A. Byles. 1987. Aspects of the biology of Virginia's sea turtles: 1979–1986. Virginia Journal of Science 38:329–336.

Keller, C. 1993. Use of fluorescent pigment for tortoise nest location. Herpetological Review 24:140–141.

Kelly, C. M. R., N. P. Barker, M. H. Villet, and D. G. Broadley. 2009. Phylogeny, biogeography and classification of the snake superfamily Elapoidea: A rapid radiation in the late Eocene. Cladistics 25:38–63.

Kelly, R. J. 1996. Review of safety guidelines for peroxidizable organic chemicals. Chemical Health and Safety 3:28–36.

Kennett, R. 1992. A new trap design for catching freshwater turtles. Wildlife Research 19:443–445.

Kennett, R. M., and A. Georges. 1990. Habitat utilization and its relationship to growth and reproduction of the eastern long-necked turtle, *Chelodina longicollis* (Testudinata: Chelidae), from Australia. Herpetologica 46:22–33.

Kenney, R. D., and G. P. Scott. 1981. Calibration of the Beechcraft AT-11 forward observation bubble for population estimation purposes. Chap. III, pp. 1–11. In Cetacean and Turtle Assessment Program (CETAP), University of Rhode Island, *A Characterization of Marine Mammals and Turtles in the Mid- and North-Atlantic Areas of the US Outer Continental Shelf, Annual Report for 1979*. Prepared for the Bureau of Land Management, Washington, DC. Ref. no. AA551-CT8-48.

Keogh, J. S. 1998. Molecular phylogeny of elapid snakes and a consideration of their biogeographic history. Biological Journal of the Linnean Society 63:177–203.

Keogh, J. S. 1999. Evolutionary implications of hemipenial morphology in the terrestrial Australian elapid snakes. Zoological Journal of the Linnean Society 125:239–278.

Kerford, M. R., A. J. Wirsing, M. R. Heithaus, and L. M. Dill. 2008. Danger on the rise: Diurnal tidal state mediates an exchange of food for safety by the Bar-bellied Sea Snake *Hydrophis elegans*. Marine Ecology Progress Series 358:289–294.

Kermeen, Q., and P. A. Lemnell. 2000. On the use of camera traps to study crocodilian nesting behavior. Crocodile Specialist Group Newsletter 19(3):17–19.

Khan, M. S. 2002. *A Guide to the Snakes of Pakistan*. Chimaira, Frankfurt am Main, Germany.

King F. W. 1989. Conservation and management. Pp. 216–229. In C. A. Ross (ed.), *Crocodiles and Alligators*. Facts on File, New York.

King, F. W., and R. L. Burke (eds.). 1989. *Crocodilian, Tuatara, and Turtle Species of the World: A Taxonomic and Geographic Reference*. Association of Systematics Collections, Washington, DC.

King, M. 1977. The evolution of sex chromosomes in lizards. Pp. 55–60. In J. H. Calaby and C. H. Tyndale-Biscoe (eds.), *Reproduction and Evolution*. Australian Academy of Science, Canberra City, Australian Capital Territory, Australia.

King, M. B., and D. Duvall. 1984. Noose tube: A lightweight, sturdy, and portable snake restraining apparatus for field and laboratory use. Herpetological Review 15:109.

King, R. B. 1986. Population ecology of the Lake Erie Water Snake, *Nerodia sipedon insularum*. Copeia 1986:757–772.

King, R. B. 1989. Sexual dimorphism in snake tail length: Sexual selection, natural selection, or morphological constraint? Biological Journal of the Linnean Society 38:133–154.

Kingsbury, B. A., and C. J. Coppola. 2000. Hibernacula of the Copperbelly Water Snake (*Nerodia erythrogaster neglecta*) in Southern Indiana and Kentucky. Journal of Herpetology 34:294–298.

Kjoss, V. A., and J. A. Litvaitis. 2001. Comparison of 2 methods to sample snake communities in early successional habitats. Wildlife Society Bulletin 29:153–157.

Klauber, L. M. 1931. A statistical survey of the snakes of the southern border of California. Bulletins of the Zoological Society of San Diego 8:1–93.

Klauber, L. M. 1932. Amphibians and reptiles observed enroute to Hoover Dam. Copeia 1932:118–128.

Klauber, L. M. 1939. Studies of reptile life in the arid southwest. Bulletins of the Zoological Society of San Diego 14:1–100.

Klauber, L. M. 1943. I. Tail-length differences in snakes, with notes on sexual dimorphism and the coefficient of divergence. II. A graphic method of showing relationships. Bulletins of the Zoological Society of San Diego, no. 18:1–76.

Klauber, L. M. 1956. *Rattlesnakes: Their Habits, Life Histories, and Influence on Mankind*. Vols. 1, 2. University of California Press, Berkeley, California.

Klauber, L. M. 1972. *Rattlesnakes: Their Habits, Life Histories and Influence on Mankind*. Vols. 1, 2. 2nd ed. University of California Press, Los Angeles, California.

Klima, E. F., G. R. Gitschlag, and M. L. Renaud. 1988. Impacts of the explosive removal of offshore petroleum platforms on sea turtles and dolphins. Marine Fisheries Review 50(3):33–42.

Klinger, R. C., R. H. George, and J. A. Musick. 1997. A bone biopsy technique for determining age and growth in sea turtles. Herpetological Review 28:31–32.

Klinger, R. C., and J. A. Musick. 1992. Annular growth layers in juvenile Loggerhead Turtles (*Caretta caretta*). Bulletin of Marine Science 51:224–230.

Klinger, R. C., and J. A. Musick. 1995. Age and growth of Loggerhead *Turtles (Caretta caretta) from Chesapeake Bay. Copeia* 1995:204–209.

Klymkowsky, M. W., and J. Hanken. 1991. Whole-mount staining of *Xenopus* and other vertebrates. Pp. 419–441. In B. K. Kay and H. B. Peng (eds.), Xenopus laevis: *Practical Uses in Cell and Molecular Biology*. Methods in Cell Biology, Vol. 36. Academic Press, San Diego, California.

Knapp, C. R. 2001. Status of a translocated *Cyclura* iguana colony in the Bahamas. Journal of Herpetology 35:239–248.

Knutson, M. G., J. R. Sauer, D. A. Olsen, M. J. Mossman, L. M. Hemesath, and M. J. Lannoo. 1999. Effects of landscape composition and wetland fragmentation on frog and toad abundance and species richness in Iowa and Wisconsin, U.S.A. Conservation Biology 13:1437–1446.

Kobayashi, M. 2000. An analysis of the growth based on the size and age distributions of the Hawksbill Sea Turtle inhabiting Cuban waters. Japanese Journal of Veterinary Research 48:129–135.

Kofron, C. P. 1989. A simple method for capturing large Nile Crocodiles. African Journal of Ecology 27:183–189.

Köhler, G. 2003. *Reptiles of Central America*. Herpeton, Offenbach, Germany.

Köhler, G., M. Veselý, and E. Greenbaum. 2006. *The Amphibians and Reptiles of El Salvador*. Krieger, Malabar, Florida.

Koper, N., and R. J. Brooks. 1998. Population-size estimators and unequal catchability in Painted Turtles. Canadian Journal of Zoology 76:458–465.

Köppl, C., G. A. Manley, and B. M. Johnstone. 1990. Peripheral auditory processing in the Bobtail Lizard *Tiliqua rugosa*. V. Seasonal effects of anaesthesia. Journal of Comparative Physiology A 167:139–144.

Kramer, M. 1986. Field studies on a freshwater Florida turtle, *Pseudemys nelsoni*. Pp. 29–34. In L. C. Drickamer (ed.), *Behavioral Ecology and Population Biology. Readings from the 19th International Ethological Conference 4, 1985: Toulouse, France*. Privat, Toulouse, France.

Krebs, C. J. 1989. *Ecological Methodology*. Harper Row, New York.

Kremen, C., A. Cameron, A. Moilanen, S. J. Phillips, C. D. Thomas, H. Beentje, J. Dransfield, B. L. Fisher, F. Glaw, T. J. Good, G. J. Harper, R. J. Hijmans, D. C. Lees, E. Louis, Jr., R. A. Nussbaum, C. J. Raxworthy, A. Razafimpahanana, G. E. Schatz, M. Vences, D. R. Vietes, P. C. Wright, and M. L. Zjhra. 2008. Aligning conservation priorities across taxa in Madagascar with high-resolution planning tools. Science 320:222–226.

Kristan, W. B., III, and W. I. Boarman. 2003. Spatial pattern of risk of common raven predation on desert tortoises. Ecology 84:2432–2443.

Kropach, C. 1971. Sea snake (*Pelamis platurus*) aggregations on slicks in Panama. Herpetologica 27:131–135.

Krysko, K. L. 2002. Seasonal activity of the Florida Kingsnake *Lampropeltis getula floridana* (Serpentes: Colubridae), in southern Florida. American Midland Naturalist 148:102–114.

Krzysik, A. J. 1998. Geographic information systems, landscape ecology, and spatial modeling. Pp. 404–428. In M. J. Lannoo (ed.), *Status and Conservation of Midwestern Amphibians*. University of Iowa Press, Iowa City, Iowa.

Kuch, U., M. Pfenninger, and A. Bahl. 1999. Laundry detergent effectively preserves amphibian and reptile blood and tissue for DNA isolation. Herpetological Review 30:80–82.

Kuchling, G. 1989. Assessment of ovarian follicles and oviduct eggs by ultra-sound scanning in live freshwater turtles, *Chelodina oblonga*. Herpetologica 45:89–94.

Kuchling, G. 1998. How to minimize risk and optimize information gain in assessing reproductive condition and fecundity of live female chelonians. Chelonian Conservation and Biology 3:118–123.

Kuchling, G. 1999. *The Reproductive Biology of the Chelonia*. Springer, Berlin, Germany.

Kuchling, G., and G. Garcia. 2003. Pelomedusidae, freshwater turtles. Pp. 956–960. In S. M. Goodman and J. P. Benstead. (eds.), *The Natural History of Madagascar*. University of Chicago Press, Chicago, Illinois.

Kuchling, G., and R. A. Mittermeier. 1993. Status and exploitation of the Madagascan Big-Headed Turtle, *Erymnochelys madagascariensis*. Chelonian Conservation and Biology 1:13–18.

Kuhnz, L. A., R. K. Burton, P. N. Slattery, and J. M. Oakden. 2005. Microhabitats and population densities of California legless lizards, with comments on effectiveness of various techniques for estimating numbers of fossorial reptiles. Journal of Herpetology 39:395–402.

Kulikova, G. S., D. V. Semenov, and G. I. Shenbrot. 1984. Quantitative characteristics of the population of the psammophilous lizards of the deserts of central Turan. P. 325. In *Abstracts of Papers, VIII Soviet Zoogeography Conference, Leningrad, 6–8 February, 1983*. Moscow. Russia. [in Russian].

Kunz, T. H. 2001. Seeing in the dark: Recent technological advances for the study of free-ranging bats. Bat Research News 42:91.

Kushlan J. A., and M. S. Kushlan. 1980. Everglades alligator nests: Nesting sites for marsh reptiles. Copeia 1980:930–932.

Laake, J. L., S. T. Buckland, D. R. Anderson, and K. P. Burnham. 1993. *Distance User's Guide, version 2.0*. Colorado Cooperative Fish and Wildlife Research Unit, Colorado State University, Fort Collins, Colorado.

Laakkonen, J., R. N. Fisher, and T. J. Case. 2001. Effect of land cover, habitat fragmentation and ant colonies on the distribution and abundance of shrews in southern California. Journal of Animal Ecology 70:776–788.

Lading, E. Ak., R. B. Stuebing, and H. K. Voris. 1991. A population size estimate of the Yellow-lipped Sea Krait, *Laticauda colubrina,* on Kalampunian Damit Island, Sabah, Malaysia. Copeia 1991:1139–1142.

Lagler, K. F. 1943. Methods of collecting freshwater turtles. Copeia 1943:21–25.

Lamar, W. W., and F. Medem. 1982. Notes on the chelid turtle *Phrynops rufipes* in Colombia. Salamandra 18:305–321.

Lamb, T., R. W. Gaul, Jr., M. L. Tripp, J. M. Horton, and B. W. Grant. 1998. A herpetofaunal inventory of the lower Roanoke River floodplain. Journal of the Elisha Mitchell Scientific Society 114:43–55.

Lance, V. A. 1989. Reproductive cycle of the American Alligator. American Zoologist 29:999–1018.

Lance, V. A., N. Valenzuela, and P. von Hilldebrand. 1992. A hormonal method to determine the sex of hatchling giant river turtles, *Podocnemis expansa*: Application to endangered species research. American Zoologist 32:16A.

Lancia, R. A., J. D. Nichols, and K. H. Pollock. 1994. Estimating the number of animals in wildlife populations. Pp. 215–253. In T. A. Bookhout (ed.), *Research and Management Techniques for Wildlife and Habitats*. 5th ed. Wildlife Society, Bethesda, Maryland.

Lang, J. W. 1976. An index to recent literature on the American alligator. In *E. A. McIlhenny's The Alligator's Life History*. Facsimile Reprints in Herpetology, Society for the Study of Amphibians and Reptiles.

Lang, R. de, and G. Vogel. 2005. *The Snakes of Sulawesi*. Chimaira, Frankfurt am Main, Germany.

Langen, T. A., A. Machniak, E. K. Crowe, C. Mangan, D. F. Marker, N. Liddle, and B. Roden. 2007. Methodologies for surveying herpetofauna mortality on rural highways. Journal of Wildlife Management 71:1361–1368.

Langkilde, T., and R. Shine. 2004. Competing for crevices: Interspecific conflict influences retreat-site selection in montane lizards. Oecologia 140:684–691.

Larsen, K. W., and P. T. Gregory. 1989. Population size and survivorship of the Common Garter Snake, *Thamnophis sirtalis*, near the northern limits of its distribution. Holarctic Ecology 12: 81–86.

Lawrence, K., and O. F. Jackson. 1983. Alphaxalone/alphadolone anaesthesia in reptiles. Veterinary Record 112:26–28.

Lawton, M. P. C. 1999. Pain management after surgery. *North American Veterinary Conference Proceedings* 1999:782.

Layfield, J. A., D. A. Galbraith, and R. J. Brooks. 1988. A simple method to mark hatchling turtles. Herpetological Review 19:78–79.

Leakey, R., and R. Lewin. 1995. *The Sixth Extinction: Patterns of Life and the Future of Humankind*. Anchor, Random House, New York.

Le Berre, M. 1989. *Faune du Sahara 1. Poissons-Amphibiens-Reptiles*. Lechevalier-R. Chaubaud, Paris, France.

Lebreton, J.-D., K. P. Burnham, J. Clobert, and D. R. Anderson. 1992. Modeling survival and testing biological hypotheses using marked animals: A unified approach with case studies. Ecological Monographs 62:67–118.

LeBuff, C. R., Jr., and P. D. Hagan. 1978. The role of aerial surveys in estimating nesting populations of the loggerhead turtle. Pp. 31–33. In *Proceedings of the Florida and Interregional Conference on Sea Turtles, 24–25 July 1976, Jensen Beach, Florida*. Florida Marine Research Publications no. 33.

Lee, J. C. 1996. *The Amphibians and Reptiles of the Yucatán Peninsula*. Comstock, Cornell University Press, Ithaca, New York.

Lee, W. L., B. M. Bell, and J. F. Sutton. 1982. *Guidelines for Acquisition and Management of Biological Specimens*. Association of Systematics Collections, Lawrence, Kansas.

Lefsky, M. A., W. B. Cohen, G. G. Parker, and D. J. Harding. 2002. Lidar remote sensing for ecosystem studies. Bioscience 52:19–30.

Legler, J. M. 1960a. A simple and inexpensive device for trapping aquatic turtles. *Proceedings of the Utah Academy of Sciences, Arts, and Letters* 37:63–66.

Legler, J. M. 1960b. Natural history of the ornate box turtle, *Terrapene ornata ornata* Agassiz. University of Kansas Publications, Museum of Natural History 11:527–669.

Legler, J. M. 1977. Stomach flushing: A technique for chelonian dietary studies. *Herpetologica* 33:281–284.

Legler, J. M. 1978. Observations on behavior and ecology in an Australian turtle, *Chelodina expansa* (Testudines: Chelidae). Canadian Journal of Zoology 56:2449–2453.

Legler, J. M., and J. Cann. 1980. A new genus and species of chelid turtle from Queensland, Australia. Contributions in Science Natural History Museum of Los Angeles County no. 324:1–18.

Lehr, E. 2002. *Amphibien und Reptilien in Peru*. Natur und Tier, Münster, Germany.

Lehr, E., G. Köehler, and B. Streit. 2002. The herpetofauna of central Peru along a transect from the Pacific Coast to the high Andes (Amphibia et Reptilia). Faunistische Abhandlungen 22:361–392. [in German]

Leick, A. 1995. *GPS Satellite Surveying*. 2nd ed. Wiley, New York.

Leiden, Y. A., M. E. Dorcas, and J. W. Gibbons. 1999. Herpetofaunal diversity in coastal plain communities of South Carolina. Journal of the Elisha Mitchell Scientific Society 115:270–280.

Lemen, C. A., and P. W. Freeman. 1985. Tracking mammals with fluorescent pigments: A new technique. Journal of Mammalogy 66:134–136.

Lemen, C. A., and H. K. Voris. 1981. A comparison of reproductive strategies among marine snakes. Journal of Animal Ecology 50:89–101.

Lemkau, P. J. 1970. Movements of the Box Turtle, *Terrapene c. carolina* (Linnaeus), in unfamiliar territory. Copeia 1970:781–783.

Lemos-Espinal, J. A., and H. M. Smith. 2007. *Anfibios y Reptiles del Estado de Coahuila, México*. Universidad Nacional Autónoma de México, México, D. F.

Lemus, D. A. 1967. Contribución al estudio de la embriología de reptiles Chilenos. II. Tabla de desarrollo de la lagartija vivípara *Liolaemus gravenhorti* (Reptilia-Squamata-Iguanidae). Biológica 40:39–61.

Lemus, D., J. Illanes, M. Fuenzalida, Y. Paz De La Vega, and M. Garcia. 1981. Comparative analysis of the development of the lizard, *Liolaemus tenuis tenuis*. II. A series of normal postlaying stages in embryonic development. Journal of Morphology 169:337–349.

Leopold, A. 1987. *A Sand County Almanac and Sketches Here and There*. Reprint ed. Oxford University Press, New York.

Leslie, A. J. 1997. The Ecology and Physiology of the Nile Crocodile, *Crocodylus niloticus,* in Lake St. Lucia, Kwazulu/Natal, South Africa. Unpubl. Ph.D. dissert., Drexel University, Philadelphia, Pennsylvania.

Letham, L. 2001. *GPS Made Easy: Using Global Positioning Systems in the Outdoors*. 3rd ed. Mountaineers Books, Seattle, Washington.

Lettink, M., and A. Cree. 2007. Relative use of three types of artificial retreats by terrestrial lizards in grazed coastal shrubland, New Zealand. Applied Herpetology 4:227–243.

Lewis, J., A. Chevallier, M. Kieny, and L. Wolpert. 1981. Muscle nerve branches do not develop in chick wings devoid of muscle. Journal of Embryology and Experimental Morphology 64:211–232.

Lewis, R. J. 2004. *Sax's Dangerous Properties of Industrial Materials*. 11th ed. Vols. 1–3. John Wiley, New York.

Lewis, T. L., and C. A. Faulhaber. 1999. Home ranges of Spotted Turtles (*Clemmys guttata*) in southwestern Ohio. Chelonian Conservation and Biology 3:430–434.

Lewis, T. L., and J. Ritzenthaler. 1997. Characteristics of hibernacula use by Spotted Turtles, *Clemmys guttata,* in Ohio. Chelonian Conservation and Biology 2:611–615.

Licht, P. 1984. Reptiles. Pp. 206–282. In G. E. Lamming (ed.), *Marshall's Physiology of Reproduction*. 4th ed. Churchill Livingstone, Edinburgh, Scotland.

Lieberman, S. S. 1986. Ecology of the leaf litter herpetofauna of a Neotropical rain forest: La Selva, Costa Rica. Acta Zoologica Mexicana 15:1–72.

Lillywhite, H. B. 1982. Tracking as an aid in ecological studies of snakes. Pp. 181–191. In N. J. Scott, Jr. (ed.), *Herpetological Communities*. U.S. Department of the Interior, Fish and Wildlife Service, Wildlife Research Report 13.

Lillywhite, H. B., C. M. Sheehy, III, and F. Zaidan. 2008. Pitviper scavenging at the intertidal zone: An evolutionary scenario for invasion of the sea. BioScience 58:947–955.

Lillywhite, H. B., and A. W. Smits. 1992. The cardiovascular adaptations of viperid snakes. Pp. 143–153. In J. A. Campbell and E. D. Brodie, Jr. (eds.), *Biology of the Pitvipers*. Selva, Tyler, Texas.

Limpus, C. J. 1984. Identification of ovarian follicles and oviducal eggs by cloacal examination of live Australian freshwater crocodiles, *Crocodylus johnstoni*. Australian Wildlife Research 11:203–204.

Limpus, C. J., J. Parmenter, and D. J. Limpus. 2002. The status of the Flatback Turtle, *Natator depressus,* in eastern Australia. Pp. 140–142. In A. Mosier, A. Foley, and B. Brost (compilers), *Proceedings of the Twentieth Annual Symposium on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFSC-477. National Marine Fisheries Service, Miami, Florida.

Limpus, C. J., and P. C. Reed. 1985. The green turtle, *Chelonia mydas,* in Queensland: A preliminary description of the population structure in a coral reef feeding ground. Pp. 47–52. In G. Grigg, R. Shine, and H. Ehmann (eds.), *Biology of Australasian Frogs and Reptiles*. Royal Zoological Society of New South Wales and Surrey Beatty, Shippping Norton, New South Wales, Australia.

Lindeman, P. V. 1996. Distribution, relative abundance, and basking ecology of the Razorback Musk Turtle, *Kinosternon carinatum,* in the Pearl and Pascagoula river drainages. Herpetological Natural History 4:23–34.

Lindeman, P. V. 1997. A comparative spotting-scope study of the distribution and relative abundance of River Cooters (*Pseudemys concinna*) in western Kentucky and southern Mississippi. Chelonian Conservation Biology 2:378–383.

Lindeman, P. V. 1998. Of deadwood and Map Turtles (*Graptemys*): An analysis of species status for five species in three river drainages using replicated spotting-scope counts of basking turtles. Chelonian Conservation and Biology 3:137–141.

Lindeman, P. V. 1999. Surveys of basking Maps Turtles *Graptemys* spp. in three river drainages and the importance of deadwood abundance. Biological Conservation 88:33–42.

Liner, E. A., and G. Casas-Andreu. 2008. *Standard Spanish, English, and Scientific Names of the Amphibians and Reptiles of Mexico.* 2nd ed. Society for the Study of Amphibians and Reptiles, Herpetological Circular no. 38

Linhart, S. B., and F. F. Knowlton. 1975. Determining the relative abundance of coyotes by scent station lines. Wildlife Society Bulletin 3:119–124.

Link, W. A. 2003. Nonidentifiability of population size from capture-recapture data with heterogeneous detection probabilities. Biometrics 59:1123–1130.

Link, W. A. 2004. Individual heterogeneity and identifiability in capture-recapture models. Animal Biodiversity and Conservation 27:87–91.

Linnaeus, C. 1758. *Systema Naturae.* 10th ed. Laurentius Salvius, Stockholm, Sweden.

Livezey, R. L. 1958. Procaine hydrochloride as a killing agent for reptiles and amphibians. Herpetologica 13:280.

Lloyd, M. L. 1999. Crocodilian anesthesia. Pp. 205–216. In R. E. Miller, M. E. Fowler, and S. Donley (eds.), *Zoo and Wild Animal Medicine: Current Therapy.* Saunders, Philadelphia, Pennsylvania.

Lloyd, M., R. F. Inger, and F. W. King. 1968. On the diversity of reptile and amphibian species in a Bornean rain forest. American Naturalist 102:497–515.

Lohoefener, R. R., W. Hoggard, C. L. Rodin, K. D. Mullin, and C. M. Rogers. 1988. Distribution and relative abundance of surfaced sea turtles in the north-central Gulf of Mexico: Spring and fall 1987. Pp. 47–50. In B. A. Schroeder (ed.), *Proceedings of the Eighth Annual Workshop on Sea Turtle Conservation and Biology.* NOAA Technical Memorandum NMFS-SEFC-214. National Marine Fisheries Service, Miami, Florida.

Loiselle, B. A., C. A. Howell, C. H. Graham, J. M. Goerck, T. Brooks, K. G. Smith, and P. H. Williams. 2003. Avoiding pitfalls of using species distribution models in conservation planning. Conservation Biology 17:1591–1600.

Lomolino, M. V., B. R. Riddle, and J. H. Brown. 2006. *Biogeography.* 3rd ed. Sinauer, Sunderland, Massachusetts.

Loncke, D. J., and M. E. Obbard. 1977. Tag success, dimensions, clutch size and nesting site fidelity for the snapping turtle, *Chelydra serpentina,* (Reptilia, Testudines, Chelydridae) in Algonquin Park, Ontario, Canada. Journal of Herpetology 11:243–244.

Longmire, J. L., A. K. Lewis, N. C. Brown, J. M. Buckingham, L. M. Clark, M. D. Jones, L. J. Meincke, J. Meyne, R. L. Ratliff, F. A. Ray, R. P. Wagner, and R. K. Moyzis. 1988. Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. Genomics 2:14–24.

Loredo, I., D. V. Vuren, and M. L. Morrison. 1996. Habitat use and migration behavior of the California Tiger Salamander. Journal of Herpetology 30:285–288.

Loveridge, J. P., and D. K. Blake. 1987. Crocodile immobilization and anaesthesia. Pp. 259–267. In G. J. W. Webb, S. C. Manolis, and P. J. Whitehead (eds.), *Wildlife Management: Crocodiles and Alligators.* Surrey Beatty, Chipping Norton, New South Wales, Australia.

Lovich, J. 1988. Aggressive basking behavior in Eastern Painted Turtles (*Chrysemys picta picta*). Herpetologica 44:197–202.

Lovich, R. 2001. Phylogeography of the night lizard, *Xantusia henshawi,* in southern California: Evolution across fault zones. Herpetologica 57:470–487.

Luiselli, L. 1998. Food habits of the Pelomedusid turtle *Pelusios castaneus castaneus* in southeastern Nigeria. Chelonian Conservation and Biology 3:106–107.

Luiselli, L., and G. C. Akani. 2002. An investigation into the composition, complexity and functioning of snake communities in the mangroves of south-eastern Nigeria. African Journal of Ecology 40:220–227.

Luiselli, L., F. M. Angelici, M. Di Vittorio, A. Spinnato, and E. Politano. 2005. Analysis of a herpetofaunal community from an altered marshy area in Sicily; with special remarks on habitat use (niche breadth and overlap), relative abundance of lizards and snakes, and the correlation between predator abundance and tail loss in lizards. Contributions to Zoology 74(3). Published online: http://dpc.uba.uva.nl/ctz/vol74/nr01/art03

Lukoschek, V., and J. S. Keogh. 2006. Molecular phylogeny of sea snakes reveals a rapidly diverged adaptive radiation. Biological Journal of the Linnean Society 89:523–539.

Lukoschek, V., M. Waycott, and H. Marsh. 2007. Phylogeography of the Olive Sea Snake, *Aipysurus laevis* (Hydrophiinae) indicates Pleistocene range expansion around northern Australia but low contemporary gene flow. Molecular Ecology 16:3406–3422.

Lutterschmidt, W. I., J. J. Lutterschmidt, and H. K. Reinert. 1996. An improved timing device for monitoring pulse frequency of temperature-sensing transmitters in free-ranging animals. American Midland Naturalist 136:172–180.

Lutterschmidt, W. I., and L. A. Rayburn. 1993. Observations of feeding behavior in *Thamnophis marcianus* after surgical procedures. Journal of Herpetology 27:95–96.

Lutterschmidt, W. I., and H. K. Reinert. 1990. The effect of ingested transmitters upon the temperature preference of the Northern Water Snake, *Nerodia s. sipedon.* Herpetologica 46:39–42.

Lutterschmidt, W. I., and J. F. Schaefer. 1996. Mist netting: Adapting a technique from ornithology for sampling semi-aquatic snake populations. Herpetological Review 27:131–132.

Luxmoore, R., B. Groombridge, and S. Broad. 1988. *Significant Trade in Wildlife: A Review of Selected Species in CITES Appendix II.* Vol. 2 *Reptiles and Invertebrates.* International Union for the Conservation of Nature and Natural Resources (IUCN), Conservation Monitoring Center, Cambridge, United Kingdom.

Lyons, D. J. 1972. New animals for Maryland – Eastern Spiny Soft-Shelled Turtle. Maryland Conservationist 48(4):22–25.

Mabee, P. M. 2000. The usefulness of ontogeny in interpreting morphological characters. Pp. 84–114. In J. J. Wiens (ed.), *Phylogenetic Analysis of Morphological Data.* Smithsonian Institution Press, Washington, DC.

MacArthur, R. H. 1957. On the relative abundance of bird species. *Proceedings of the National Academy of Sciences (USA)* 43:293–295.

MacArthur, R. H., and E. O. Wilson. 1967. *The Theory of Island Biogeography.* Princeton University Press, Princeton, New Jersey.

MacCulloch, R. D., D. E. Upton, and R. W. Murphy. 1996. Trends in nuclear DNA content among amphibians and reptiles. Comparative Biochemical Physiology 113B:601–605.

Macey, J. R., N. B. Ananjeva, Y. Wang, and T. J. Papenfuss. 1997. A taxonomic reevaluation of the gekkonid lizard genus *Teratoscincus* in China. Russian Journal of Herpetology 4:8–16.

Macey, J. R., N. B. Ananjeva, Y. Wang, and T. J. Papenfuss. 2000. Phylogenetic relationships among Asian gekkonid lizards formerly of the genus *Cyrtodactylus* based on cladistic analyses of allozymic data: Monophyly of *Cyrtopodion* and *Mediodactylus.* Journal of Herpetology 34:258–265.

Macey, J. R., J. A. Schulte, II, N. B. Ananjeva, A. Larson, N. Rastergar-Pouyani, S. M. Shammakov, and T. J. Papenfuss. 1998. Phylogenetic relationships among agamid lizards of the *Laudakia caucasia* complex: Testing hypotheses of biogeographic fragmentation and an area cladogram for the Iranian plateau. Molecular Phylogenetics and Evolution 10:118–131.

Macey, J. R. Y. Wang, N. B. Ananjeva, A. Larson, and T. J. Papenfuss. 1999. Vicariant patterns of fragmentation among gekkonid lizards of the genus *Teratoscincus* produced by the Indian collision: A molecular phylogenetic perspective and an area cladogram for central Asia. Molecular Phylogenetics and Evolution 12:320–332.

MacGregor, G. A., and H. K. Reinert. 2001. The use of passive integrated transponders (PIT tags) in snake foraging studies. Herpetological Review 32:170–172.

MacKenzie, D. I. 2005a. Was it there? Dealing with imperfect detection for species presence/absence data. Australian and New Zealand Journal of Statistics 47:65–74.

MacKenzie, D. I. 2005b. What are the issues with presence-absence data for wildlife managers? Journal of Wildlife Management 69:849–860.

MacKenzie, D. I. 2006. Modeling the probability of resource use: The effect of, and dealing with, detecting a species imperfectly. Journal of Wildlife Management 70:367–374.

MacKenzie, D. I., and L. L. Bailey. 2004. Assessing the fit of site-occupancy models. Journal of Agricultural, Biological, and Environmental Statistics 9:300–318.

MacKenzie, D. I., L. L. Bailey, and J. D. Nichols. 2004. Investigating species co-occurrence patterns when species are detected imperfectly. Journal of Animal Ecology 73:546–555.

MacKenzie, D. I., and J. E. Hines. 2002–2010. *PRESENCE2-Software to Estimate Patch Occupancy and Related Parameters.* U.S. Geological Survey, Patuxent Wildlife Research Center. http://www.mbr-pwrc.usgs.gov/software/presence.shtml

MacKenzie, D. I., and W. L. Kendall. 2002. How should detection probability be incorporated into estimates of relative abundance? Ecology 83:2387–2393.

MacKenzie, D. I., and J. D. Nichols. 2004. Occupancy as a surrogate for abundance estimation. Animal Biodiversity and Conservation 27:461–467.

MacKenzie, D. I., J. D. Nichols, J. E. Hines, M. G. Knutson, and A. B. Franklin. 2003. Estimating site occupancy, colonization, and local extinction when a species is detected imperfectly. Ecology 84:2200–2207.

MacKenzie, D. I., J. D. Nichols, G. B. Lachman, S. Droege, J. A. Royle, and C. A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. Ecology 83:2248–2255.

MacKenzie, D. I., J. D. Nichols, J. A. Royle, K. H. Pollock, L. L. Bailey, and J. E. Hines. 2006. *Occupancy Estimation and Modeling: Inferring Patterns and Dynamics of Species Occurrence*. Academic Press, San Diego.

MacKenzie, D. I., J. D. Nichols, N. Sutton, K. Kawanishi, and L. L. Bailey. 2005. Improving inference in population studies of rare species that are detected imperfectly. Ecology 86:1101–1113.

MacKenzie, D. I., and J. A. Royle. 2005. Designing occupancy studies: General advice and allocating survey effort. Journal of Applied Ecology 42:1105–1114.

MacKenzie, D. I., J. A. Royle, J. A. Brown, and J. D. Nichols. 2004. Occupancy estimation and modeling for rare and elusive populations. Pp. 149–172. In W. L. Thompson (ed.), *Sampling Rare or Elusive Species: Concepts, Designs, and Techniques for Estimating Population Parameters*. Island Press, Washington, DC.

Magnusson, W. E. 1982. Techniques of surveying for crocodilians. Pp. 389–403. In *Crocodiles: Proceedings of the 5th Annual Working Meeting of the Crocodile Specialist Group of the Species Survival Commission of the International Union for the Conservation of Nature and Natural Resources*. Gland, Switzerland.

Magnusson, W. E. 1986. The peculiarities of crocodilian population dynamics and their possible importance for management strategies. Pp. 434–442. In *Crocodiles: Proceedings of the 7th Working Meeting of the Crocodile Specialist Group of the Species Survival Commission of the IUCN*. IUCN/FUDENA, Caracas, Venezuela.

Magnusson, W. E. 1989. *Paleosuchus*. Pp. 101–109. In *Crocodiles: Their Ecology, Management, and Conservation*. Special Publication of the Crocodile Specialist Group of the Species Survival Commission of the International Union for Conservation of Nature and Natural Resources. Gland, Switzerland.

Magnusson, W. E., A. Cardoso de Lima, V. Lopes da Costa, and O. Pimentel de Lima. 1997. Growth of the turtle, *Phrynops rufipes,* in Central Amazônia, Brazil. Chelonian Conservation and Biology 2:576–581.

Magnusson, W. E., A. Cardoso de Lima, V. Lopes da Costa, and R. C. Vogt. 1997. Home range of the turtle, *Phrynops rufipes,* in an isolated reserve in central Amazônia, Brazil. Chelonian Conservation and Biology 2:494–499.

Magnusson, W. E., G. J. Caughley, and G. C. Grigg. 1978. A double-survey estimate of population size from incomplete counts. Journal of Wildlife Management 42:174–176.

Magnusson, W. E., G. C. Grigg, and J. A. Taylor. 1980. An aerial survey of potential nesting areas of *Crocodylus porosus* on the west coast of Cape York Peninsula. Australian Wildlife Research 7:465–478.

Magnusson, W. E., and A. P. Lima. 1991. The ecology of a cryptic predator, *Paleosuchus trigonatus,* in a tropical rainforest. Journal of Herpetology 25:41–48.

Magurran, A. E. 1988. *Ecological Diversity and Its Measurement.* Princeton University Press, Princeton, New Jersey.

Mahmoud, I. Y. 1969. Comparative ecology of the kinosternid turtles of Oklahoma. Southwestern Naturalist 14:31–66.

Mahmoud, I. Y., G. L. Hess, and J. Klicka. 1973. Normal embryonic stages of the Western Painted Turtle, *Chrysemys picta bellii.* Journal of Morphology 141:269–279.

Malone J. H., and D. Laurencio. 2004. The use of polystyrene for drift fence sampling in a tropical forest. Herpetological Review 35:142–143.

Manly, B. F. J., T. L. McDonald, S. C. Amstrup, and E. V. Regehr. 2003. Improving size estimates of open animal populations by incorporating information on age. Bioscience 53:666–669.

Manne, L. L., and P. H. Williams. 2003. Building indicator groups based on species characteristics can improve conservation planning. Animal Conservation 6:291–297.

Manning, P. L. 2004. A new approach to the analysis and interpretation of tracks: Examples from the dinosauria. Pp. 93–123. In D. McIlroy (ed.), *The Application of Ichnology to Palaeoenvironmental and Stratigraphic Analysis*. Geological Society, Special Publication no. 228, London, England.

Mansfield, P., E. G. Strauss, and P. J. Auger. 1998. Using decoys to capture Spotted Turtles (*Clemmys guttata*) in water funnel traps. Herpetological Review 29:157–158.

Manthey, U. 2008. *Agamid Lizards of Southern Asia: Draconinae 1.* Chimaira, Frankfurt am Main, Germany.

Manthey, U. 2010. *Agamid Lizards of Southern Asia: Draconinae 2, Leiolepidinae.* Chimaira, Frankfurt am Main, Germany.

Mao, J.-J., G. Norval, and P. Müller. 2009. The use of vehicle-portable scanners for obtaining morphological data on small- to medium-sized reptiles. Herpetological Review 40:37–41.

Marchand, L. J. 1945. Water goggling: A new method for the study of turtles. Copeia 1945:37–40.

Margoluis, R., and N. Salafsky. 1998. *Measures of Success: Designing, Managing, and Monitoring Conservation and Development Projects.* Island Press, Washington, DC.

Markwell, T. J. 1997. Video camera count of burrow-dwelling fairy prions, sooty shearwaters, and tuatara on Takapourewa (Stephens Island), New Zealand. New Zealand Journal of Zoology 24:231–237.

Marques, O. A. V. 1998. Composição Faunística, História Natural e Ecologia de Serpentes da Mata Atlântica, na Região da Estação Ecológica Juréia-Itatins, São Paulo, SP. Unpubl. Ph.D. dissert., Universidade de São Paulo, São Paulo, Brasil.

Marsh, D. M., and M. A. Goicochea. 2003. Monitoring terrestrial salamanders: Biases caused by intense sampling and choice of cover objects. Journal of Herpetology 37:460–466.

Marsh, H., and W. K. Saalfeld. 1989. Aerial surveys of sea turtles in the northern Great Barrier Reef Marine Park. Australian Wildlife Research 16:239–249.

Marsh, H., and D. F. Sinclair. 1989. An experimental evaluation of dugong and sea turtle aerial survey techniques. Australian Wildlife Research 16:639–650.

Marshall, B., and F. I. Woodward (eds.). 1985. *Instrumentation for Environmental Physiology.* Cambridge University Press, Cambridge, England.

Martin, B. J. 1995. Evaluation of hypothermia for anesthesia in reptiles and amphibians. ILAR Journal 37:186–190.

Martin, D. J., and D. B. Fagre. 1988. Field evaluation of a synthetic coyote attractant. Wildlife Society Bulletin 16:390–396.

Martin, P. L., and J. N. Layne. 1987. Relationship of Gopher Tortoise body size to burrow size in a southcentral Florida population. Florida Scientist 50:264–267.

Marton-Lefèvre, J. 2010. Biodiversity is our life. Science 321:1179.

Mattox, N. T. 1936. Annular rings in the long bones of turtles and their correlation with size. Transactions of the Illinois State Academy of Science 28:255–256.

Mauger, D., and T. P. Wilson. 1999. Population characteristics and seasonal activity of *Sistrurus catenatus catenatus* in Will County, Illinois: Implications for management and monitoring. Pp. 110–124. In B. Johnson and M. Wright (eds.), *Second International Symposium and Workshop on Conservation of the Eastern Massasauga Rattlesnake,* Sistrurus catenatus catenatus*: Population and Habitat Management Issues in Urban, Bog, Prairie, and Forested Ecosystems, 1998*. Toronto Zoo, Toronto, Ontario, Canada.

Mauldin, R. E., and R. M. Engeman. 1999. A novel snake restraint device. Herpetological Review 30:158.

May, P. G., T. M. Farrell, S. T. Heulett, M. A. Pilgrim, L. A. Bishop, D. J. Spence, A. M. Rabatsky, M. G. Campbell, A. D. Aycrigg, and W. E. Richardson II. 1996. Seasonal abundance and activity of a rattlesnake (*Sistrurus miliaris barbouri*) in central Florida. Copeia 1996:389–401.

May, R. M. 1975. Patterns of species abundance and diversity. Pp. 81–120. In M. L. Cody and J. M. Diamond (eds.), *Ecology and Evolution of Communities*. Belknap, Harvard University Press, Cambridge, Massachusetts.

May, R. M. 1988. How many species are there on Earth? Science (USA) 241:1441–1449.

May, R. M. 1990. How many species? Philosophical Transactions of the Royal Society of London B 330:293–304.

Mayor, P. A., P. T. Plotkin, J. R. Spotila, and F. V. Paladino. 1998. Leatherback Turtle nesting on the Pacific coast of Costa Rica. P. 243. In S. P. Epperly and J. Braun (compilers), *Proceedings of the Seventeenth Annual Sea Turtle Symposium*. U.S. Department of Commerce. NOAA Technical Memorandum NMFS-SEFSC-415. National Marine Fisheries Service, Miami, Florida.

Mayr, E. 1963. *Animal Species and Evolution*. Belknap Press, Harvard University Press, Cambridge, Massachusetts.

Mazerolle, M. J., L. L. Bailey, W. L. Kendall, J. A. Royle, S. J. Converse, and J. D. Nichols. 2007. Making great leaps forward: Accounting for detectability in herpetological field studies. Journal of Herpetology 41:672–689.

Mazzotti, F. J. 1983. The Ecology of *Crocodylus acutus* in Florida. Unpubl. Ph.D. dissert., Pennsylvania State University, University Park, Pennsylvania.

Mazzotti, F. J, and L. A. Brandt. 1988. A method of live-trapping crocodiles. Herpetological Review 19:40–41.

McCauley, D. J., F. Keesing, T. P. Young, B. F. Allan, and R. M. Pringle. 2006. Indirect effects of large herbivores on snakes in an African savanna. Ecology 87:2657–2663.

McCoy, E. D., and H. R. Mushinsky. 1994. Effects of fragmentation on the richness of vertebrates in the Florida scrub habitat. Ecology 75:446–457.

McCoy, E. D., and H. R. Mushinsky. 1995. *The Demography of* Gopherus polyphemus *(Daudin) in Relation to Size of Available Habitat*. Florida Game and Fresh Water Fish Commission, Nongame Wildlife Program Project Report GFC-86-013 (submitted 1988), Tallahassee, Florida. http://research.myfwc.com/publications/publication_info. asp?id=47223

McCoy, E. D., and H. R. Mushinsky. 2007. Estimates of minimum patch size depend upon the method of estimation and the condition of the habitat. Ecology 88:1401–1407.

McCoy, E. D., H. R. Mushinsky, and D. S. Wilson. 1993. Patterns in the compass orientation of Gopher Tortoise burrows at different spatial scales. Global Ecology and Biogeography Letters 3:33–40.

McCoy, M. 1985. I bought 200 poisonous snakes from children. Geo 7:90–101.

McCranie, J. R., J. H. Townsend, and L. D. Wilson. 2006. *The Amphibians and Reptiles of the Honduran Mosquitia*. Krieger, Malabar, Florida.

McCullagh, P., and J. A. Nelder. 1989. *Generalized Linear Models*. 2nd ed. Chapman Hall, New York.

McDaniel, C. J., L. B. Crowder, and J. A. Priddy. 2000. Spatial dynamics of sea turtle abundance and shrimping intensity in the U.S. Gulf of Mexico. Conservation Ecology 4(1): article 15. http://www.consecol.org/vol4/iss1/art15/

McDaniel, J., and L. Hord. 1990. Specialized equipment and techniques used in alligator management and research. Pp. 20–38. In *Crocodiles: Proceedings of the 10th Working Meeting of the Crocodile Specialist Group of the Species Survival Commission of IUCN-The World Conservation Union*. Vol. 2. IUCN-The World Conservation Union, Gland, Switzerland.

McDiarmid, R. W. 1994. Preparing amphibians as scientific specimens. Pp. 289–297. In W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster (eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Smithsonian Institution Press, Washington, DC.

McDonald, D. L., and P. H. Dutton. 1996. Use of PIT tags and photoidentification to revise remigration estimates of Leatherback Sea Turtles (*Dermochelys coriacea*) nesting in St. Croix, US Virgin Islands, 1979–1995. Chelonian Conservation and Biology 2:148–152.

McDonald, D., P. Dutton, R. Brandner, and S. Basford. 1996. Use of pineal spot ("pink spot") photographs to identify Leatherback Turtles. Herpetological Review 27:11–12.

McDonald, H. S. 1976. Methods for the physiological study of reptiles. Pp. 19–126. In C. Gans and W. R. Dawson (eds.), *Biology of the Reptilia* Vol. 5. *Physiology A*. Academic Press, New York.

McGinnis, S. M. 1967. The adaptation of biotelemetry techniques to small reptiles. Copeia 1967:472–473.

McIlhenny, E. A. 1935. *The Alligator's Life History.* Christopher, Boston, Massachusetts.

McIntosh, R. P. 1967. An index of diversity and the relation of certain concepts to diversity. Ecology 48:392–404.

Means, D. B. 2009. Effects of rattlesnake roundups on the Eastern Diamondback Rattlesnake (*Crotalus adamanteus*). Herpetological Conservation and Biology 4:132–141.

Medem, F. 1981. *Los Crocodylia de Sur America*. Vol. I. *Los Crocodylia de Colombia*. Ministaerio de Educacion Nacional, Colciencias, Bogotá, Colombia.

Medem, F. 1983. *Los Crocodylia de Sur America*. Vol. II. *Venezuela, Trinidad, Tobago, Guyana, Suriname, Guayana Francesa, Ecuador, Perú, Bolivia, Brasil, Paraguay, Argentina, Uruguay*. Universidad Nacional de Colombia y Fondo Colombiano de Investigaciones Cientificas y Proyectos Especiales "Francisco Jose de Caldas", Colciencias, Bogotá, Colombia.

Medica, P. A., G. A. Hoddenbach, and J. R. Lannom, Jr. 1971. *Lizard Sampling Techniques*. Rock Valley Miscellaneous Publications no. 1. 55 pp.

Mendelson, J. R., III. 1992. Frequency of tail breakage in *Coniophanes fissidens* (Serpentes: Colubridae). Herpetologica 48:448–455.

Mendelson, J. R., III, and W. B. Jennings. 1992. Shifts in the relative abundance of snakes in a desert grassland. Journal of Herpetology 26:38–45.

Menkins, G. E., Jr., and S. H. Anderson. 1988. Estimation of small-mammal population size. Ecology 69:1952–1959.

Merchant-Larios, H. 1999. Determining hatchling sex. Pp. 130–135. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, and M. Donnelly (eds.), *Research and Management Techniques for the Conservation of Sea Turtles*. IUCN/SSC Marine Turtle Specialist Group Publication no. 4.

Messel, H., and G. C. Vorlicek. 1989a. Growth of *Crocodylus porosus* in the wild in northern Australia. Pp. 110–137. In *Crocodiles: Their Ecology, Management, and Conservation*. Special Publication of the Crocodile Specialist Group of the Species Survival Commission of the International Union for Conservation of Nature and Natural Resources, Gland, Switzerland.

Messel, H., and G. C. Vorlicek. 1989b. Status and conservation of *Crocodylus porosus* in Australia. Pp. 138–163. In *Crocodiles: Their Ecology, Management, and Conservation*. Special Publication of the Crocodile Specialist Group of the Species Survival Commission of the International Union for Conservation of Nature and Natural Resources, Gland, Switzerland.

Messel, H., and G. C. Vorlicek. 1989c. Ecology of *Crocodylus porosus* in northern Australia. Pp. 164–183. In *Crocodiles: Their Ecology, Management, and Conservation*. Special Publication of the Crocodile Specialist Group of the Species Survival Commission of the International Union for Conservation of Nature and Natural Resources, Gland, Switzerland.

Messel, H., G. C. Vorlicek, M. Elliott, A. G. Wells, and W. J. Green. 1981. *Surveys of Tidal River Systems in the Northern Territory of Australia and Their Crocodile Populations*. Monograph 17. *Darwin and Bynoe Harbours and Their Tidal Waterways*. Pergammon, Rushcutters Bay, Australia.

Messel, H., G. C. Vorlicek, A. G. Wells, and W. J. Green. 1981. *Surveys of Tidal River Systems in the Northern Territory of Australia and their Crocodile Populations*. Monograph 1. *The Blyth-Cadell Rivers System and the Status of* Crocodylus porosus *in Tidal Waterways of Northern Australia: Methods for Analysis, and Dynamics of a Population of* C. porosus. Pergamon, Sydney, Australia.

Metts, B. S., J. D. Lanham, and K. R. Russell. 2001. Evaluation of herpetofaunal communities on upland streams and beaver-impounded streams in the Upper Piedmont of South Carolina. American Midland Naturalist 145:54–65.

Meylan, A. 1995. Estimation of population size in sea turtles. Pp. 135–138. In K. A. Bjorndal (ed.), *Biology and Conservation of Sea Turtles*. Rev. ed. Smithsonian Institution Press, Washington, DC.

Michael, D. R., I. D. Lunt, and W. A. Robinson. 2004. Enhancing fauna habitat in grazed native grasslands and woodlands: Use of artificially placed log refuges by fauna. Wildlife Research 31:65–71.

Mieres, M. M., and L. A. Fitzgerald. 2006. Monitoring and managing and the harvest of Tegu Lizards in Paraguay. Journal of Wildlife Management 70:1723–1734.

Mieres Romero, M. M. 2002. Monitoring and Managing the Harvest of tegu Lizards in Paraguay. Unpubl. Master's thesis, Texas A & M University, College Station, Texas.

Miller, D. E., and H. R. Mushinsky. 1990. Foraging ecology and prey size in the Mangrove Water Snake, *Nerodia fasciata compressicauda*. Copeia 1990:1099–1106.

Miller, J. D. 1978. A modification of Vernier calipers for measurement of convex curved surfaces. Herpetological Review 9:132–133.

Miller, J. D. 1985. Embryology of marine turtles. Pp. 269–328. In C. Gans, F. Billett, and P. F. A. Maderson (eds.), *Biology of the Reptilia*, Vol. 14. *Development A*. Wiley, New York.

Miller, L. R., and W. H. N. Gutzke. 1998. Sodium brevital as an anesthetizing agent for crotalines. Herpetological Review 29:16.

Miller, R. I., and R. G. Wiegert. 1989. Documenting completeness, species-area relations, and the species-abundance distribution of a regional flora. Ecology 70:16–22.

Mills, M. S. 2002. Ecology and Life History of the Brown Water Snake (*Nerodia taxispilota*). Unpubl. Ph. D. dissert., University of Georgia, Athens, Georgia.

Millspaugh, J. J., and J. M. Marzluff (eds.). 2001. *Radio Tracking and Animal Populations*. Academic Press, San Diego, California.

Milne T., and C. M. Bull. 2002. Characteristics of litters and juvenile dispersal in the endangered Australian skink *Tiliqua adelaidensis*. Journal of Herpetology 36:110–112.

Milstead, W. W. 1953. Ecological distribution of the lizards of the La Mota Mountain region of Trans-Pecos Texas. Texas Journal of Science 5:403–415.

Minahan, K., G. Mills, S. Hayden, and J. W. Gibbons. 2002. An assessment of hydrocarbon contamination derived from roofing material coverboards. Herpetological Review 33:36–38.

Minakami, K. 1977. Age determination of the Yellow-green Pitviper, habu by the vertebral centrum. Zoology Magazine [Dobutsugaku Zasshi] 86:82–86.

Minakami, K. 1979. An estimation of age and life-span of the genus *Trimeresurus* (Reptilia, Serpentes, Viperidae) on Amami Oshima Island, Japan. Journal of Herpetology 13:147–152.

Miniwatts Marketing Group. 2000–2010. Internet World Stats: Usage and Population Statistics. www.internetworldstats.com [accessed November 2010].

Minnich, J. E., and V. H. Shoemaker. 1970. Diet, behavior and water turnover in the Desert Iguana, *Dipsosaurus dorsalis*. American Midland Naturalist 84:496–509.

Mitchell, A. W. 1982. *Reaching the Rain Forest Roof: A Handbook of Techniques of Access and Study in the Canopy.* Leeds Philosophical and Literary Society, Leeds, England.

Mitchell, J. C., R. E. Jung Brown, and B. Bartholomew (eds.). 2008. *Urban Herpetology.* Herpetological Conservation Vol. 3. Society for the Study of Amphibians and Reptiles, Salt Lake City, Utah.

Mitchell, N. C. 1999. Effect of introduced ungulates on density, dietary preferences, home range, and physical condition of the iguana (*Cyclura pinguis*) on Anegada. Herpetologica 55:7–17.

Mockford, S. W., J. M. Wright, M. Snyder, and T. B. Herman. 1999. A non-destructive source of DNA from hatchling freshwater turtles for use in PCR base assays. Herpetological Review 30:148–149.

Moffat, L. A. 1985. Embryonic development and aspects of reproductive biology in the tuatara, *Sphenodon punctatus*. Pp. 493–521. In C. Gans, F. Billett, and P. F. A. Maderson (eds.), *Biology of the Reptilia,* Vol. 14. *Development A*. Wiley, New York.

Moler, P. E. 1992. Eastern Indigo Snake *Drymarcon corais couperi* (Holbrook). Pp. 181–186. In P. E. Moler (ed.), *Rare and Endangered Biota of Florida*. Vol. III. *Amphibians and Reptiles*. University of Florida Press, Gainesville, Florida.

Moll, D. 1976. Food and feeding strategies of the Ouachita Map Turtle (*Graptemys pseudogeographica ouachitensis*). American Midland Naturalist 96:478–482.

Moll, D. 1986. The distribution, status, and level of exploitation of the freshwater turtle, *Dermatemys mawei* in Belize, Central America. Biological Conservation 35:87–96.

Moll, D. 1990. Population sizes and foraging ecology in a tropical freshwater stream turtle community. Journal of Herpetology 24:48–53.

Moll, D. 1994. The ecology of sea beach nesting in Slider Turtles (*Trachemys scripta venusta*) from Caribbean Costa Rica. Chelonian Conservation and Biology 1:107–116.

Moll, E. O., and J. M. Legler. 1971. The life history of a neotropical slider turtle, *Pseudemys scripta* (Schoepff), in Panama. Bulletin of the Los Angeles County Museum of Natural History, Science 11:1–102.

Monfort, S. L., C. C. Schwartz, and S. K. Wasser. 1993. Monitoring reproduction in captive moose using urinary and fecal steroid metabolites. Journal of Wildlife Management 57:400–407.

Montague, J. J. 1983. Influence of water level, hunting pressure and habitat type on crocodile abundance in the Fly River drainage, Papua New Guinea. Biological Conservation 26:309–339.

Montgomery, G. G., A. S. Rand, and M. E. Sunquist. 1973. Post-nesting movements of iguanas from a nesting aggregation. Copeia 1973:620–622.

Monti, L., M. Hunter, Jr., and J. Witham. 2000. An evaluation of the artificial cover object (ACO) method for monitoring populations of the Redback Salamander *Plethodon cinereus*. Journal of Herpetology 34:624–629.

Moore, F. L. 1987. Regulation of reproductive behaviors. Pp. 505–522. In D. O. Norris and R. E. Jones (eds.), *Hormones and Reproduction in Fishes, Amphibians, and Reptiles*. Plenum, New York.

Moore, M. C., and J. Lindzey. 1992. The physiological basis of sexual behavior in male reptiles. Pp. 70–113. In C. Gans and D. Crews (eds.), *Biology of the Reptilia* Vol. 18. *Physiology F, Hormones, Brain, and Behavior.* University of Chicago Press, Chicago, Illinois.

Morales-Verdeja, S. A., and R. C. Vogt. 1997. Terrestrial movements in relation to aestivation and the annual reproductive cycle of *Kinosternon leucostomum*. Copeia.1997:123–130.

Morris, P. J., and A. C. Alberts. 1996. Determination of sex in White-throated Monitors (*Varanus albigularis*), Gila Monsters (*Heloderma suspectum*), and Beaded Lizards (*H. horridum*) using two-dimensional ultrasound imaging. Journal of Zoo and Wildlife Medicine 27:371–377.

Morris, P. J., L. A. Jackintell, and A. C. Alberts. 1996. Predicting the gender of subadult Komodo Dragons (*Varanus komodoensis*) using two-dimensional ultrasound imaging and plasma testosterone concentration. Zoo Biology 15:341–348.

Morrison, A. R. 2009. *An Odyssey with Animals: A Veterinarian's Reflections on the Animal Rights and Welfare Debate*. Oxford University Press, Oxford, England.

Morrison, M. L., W. M. Block, M. D. Strickland, B. A. Collier, and M. J. Peterson. 2008. *Wildlife Study Design*. 2nd ed. Springer, New York.

Morrison, S. A., and D. T. Bolger. 2002. Variation in a sparrow's reproductive success with rainfall: Food and predator-mediated processes. Oecologia 133:315–324.

Morton, D. B., G. M. Burghardt, and J. A. Smith. 1990. Critical anthropomorphism, animal suffering, and the ecological context. Pp. S13–S19. In S. Donnelley and K. Nolan (eds.), *Animals, Science, and Ethics*. Hastings Center Report. Vol. 20(3) Special Supplement.

Mosauer, W. 1932. Adaptive convergence in the sand reptiles of the Sahara and of California: A study in structure and behavior. Copeia 1932:72–78.

Mosauer, W. 1933. Locomotion and diurnal range of *Sonora occipitalis, Crotalus cerastes,* and *Crotalus atrox* as seen from their tracks. Copeia 1933:14–16.

Mosauer, W. 1935. The reptiles of a sand dune area and its surroundings in the Colorado desert, California: A study in habitat preference. Ecology 16:13–27.

Mosimann, J. E., and J. R. Bider. 1960. Variation, sexual dimorphism, and maturity in a Quebec population of the Common Snapping Turtle, *Chelydra serpentina*. Canadian Journal of Zoology 38:19–38.

Mourão, G. M., P. Bayliss, M. E. Coutinho, C. L. Abercrombie, and A. Arruda. 1994. Test of an aerial survey for caiman and other wildlife in the Pantanal of Brazil. Wildlife Society Bulletin 22:50–56.

Mourão, G., Z. Campos, and M. Coutinho. 1994. Aerial surveys of caiman nests in wet savannas of Brazil. Pp. 236–240. In *Crocodiles: Proceedings of the 12th Working Meeting of the Crocodile Specialist Group of the Species Survival Commission of IUCN-The World Conservation Union.* Vol. 2. IUCN- The World Conservation Union, Gland, Switzerland.

Mourão, G., M. Coutinho, R. Mauro, Z. Campos, W. Tomás, and W. Magnusson. 2000. Aerial surveys of caiman, Marsh Deer and Pampas Deer in the Pantanal wetland of Brazil. Biological Conservation 92:175–183.

Mourão, G. M., and W. E. Magnusson. 1997. Uso de levantamentos aéreos para o manejo de populações silvestres. Pp. 23–33. In C. Valladares-Padua, R. E. Bodmer, and L. Cullen, Jr. (eds.), *Manejo e Conservação da Vida Silvestre no Brasil.* MCT-CNPq, Sociedade Civil Mamirauá, Brasília, D. F., Brazil.

Mrosovsky, N., C. Baptistotte, and M. H. Godfrey. 1999. Validation of incubation duration as an index of the sex ratio of hatchling sea turtles. Canadian Journal of Zoology 77:831–835.

Mueller, G. M., G. F. Bills, and M. S. Foster (eds.). 2004. *Biodiversity of Fungi, Inventory and Monitoring Methods*. Elsevier Academic Press, New York.

Mullin, K. 2000. Estimates of sea turtle abundance from aerial surveys. Pp. 66–70. In K. A. Bjorndal and A. B. Bolten (eds.), *Proceedings of a Workshop on Assessing Abundance and Trends for In-Water Sea Turtle Populations*. NOAA Technical Memorandum NMFS-SEFSC-445. National Marine Fisheries Service, Miami, Florida.

Muñoz, M. C., and J. Thorbjarnarson. 2000. Movement of captive-released Orinoco Crocodiles (*Crocodylus intermedius*) in the Capanaparo River, Venezuela. Journal of Herpetology 34:397–403.

Murie, O. J. 1954. *A Field Guide to Animal Tracks*. Houghton Mifflin, Boston, Massachusetts.

Murphy, B. R., and D. W. Willis. 1996. *Fisheries Techniques*. American Fisheries Society, Bethesda, Maryland.

Murphy, C. G. 1993. A modified drift fence for capturing treefrogs. Herpetological Review 24:143–145.

Murphy, J. B., and B. L. Armstrong. 1978. Maintenance of rattlesnakes in captivity. University of Kansas Museum of Natural History Special Publication 3:1–40.

Murphy, J. B., and J. A. Campbell. 1987. Captive maintenance. Pp. 165–181. In R. A. Seigel, J. T. Collins, and S. S. Novak (eds.), *Snakes: Ecology and Evolutionary Biology*. Macmillan, New York.

Murphy, J. B., and K. McCloud. 2010. Reptile dealers and their price lists. Herpetological Review 41:266–281.

Murphy, J. C. 1997. *Amphibians and Reptiles of Trinidad and Tobago*. Krieger, Malabar, Florida.

Murphy, J. C. 2007. *Homalopsid Snakes: Evolution in the Mud*. Krieger, Malabar, Florida.

Murphy, J. C., H. K**.** Voris, D. R. Karns, T. Chan-ard, and K. Suvunrat. 1999. The ecology of the water snakes of Ban Tha Hin, Songkhla Province, Thailand. Natural History Bulletin of the Siam Society 47:129–147.

Murphy, R. W., L. A. Lowcock, C. Smith, I. S. Darevsky, N. Orlov, R. D. MacCulloch, and D. E. Upton. 1997. Flow cytometry in biodiversity surveys: Methods, utility, and constraints. Amphibia-Reptilia 18:1–13.

Murphy, T. M., Jr., and T. T. Fendley. 1974. A new technique for live trapping of nuisance alligators. Pp. 308–311. In *Proceedings of the Twenty–seventh Annual Conference, Southeastern Association of Game and Fish Commissioners, 1973*.

Murray, M. J. 1996. Cardiology and circulation. Pp. 95–104. In D. R. Mader (ed.), *Reptile Medicine and Surgery*. Saunders, Philadelphia, Pennsylvania.

Mushinsky, H. R., and J. J. Hebrard. 1977. The use of time by sympatric water snakes. Canadian Journal of Zoology 55:1545–1550.

Mushinsky, H. R., J. J. Hebrard, and M. G. Walley. 1980. The role of temperature on the behavioral and ecological associations of sympatric water snakes. Copeia 1980:744–754.

Mushinsky, H. R., and E. D. McCoy. 1994. Comparison of Gopher Tortoise populations on islands and on the mainland in Florida. Pp. 39–47. In R. B. Bury and D. J. Germano (eds.), *Biology of North American Tortoises*. U.S. Department of the Interior, National Biological Survey, Fish and Wildlife Research no. 13. Washington, DC.

Mushinsky, H. R., and A. H. Savitzky (with contributions from E. Brodie, Jr., W. Brown, J. Campbell, K. Enge, L. Fitzgerald, H. Greene, P. Gregory, J. Jensen, P. Moler, C. Painter, A. Price, and W. Timmerman). 2006. *Position of The American Society of Ichthyologists and Herpetologists concerning Rattlesnake Conservation and Roundups*. American Society of Ichthyologists and Herpetologists. Published online: http://www.asih.org/

Musick, J. A., D. Barnard, and J. A. Keinath. 1992. Prediction of trawl fishery impacts on sea turtles: A model. Pp. 78–82. In M. Salmon and J. Wyneken (compilers), *Proceedings of the Eleventh Annual Workshop on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFSC-302. National Marine Fisheries Service, Miami, Florida.

Musick, J. A., D. E. Barnard, and J. A. Keinath. 1994. Aerial estimates of seasonal distribution and abundance of sea turtles near the Cape Hatteras faunal barrier. Pp. 121–123. In B. A. Schroeder and B. E. Witherington (compilers), *Proceedings of the Thirteenth Annual Symposium on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFSC-341. National Marine Fisheries Service, Miami, Florida.

Muths, E., A. L. Gallant, E. H. Campbell Grant, W. A. Battaglin, D. E. Green, J. S. Staiger, S. C. Walls, M. S. Gunzburger, and R. F. Kearney. 2006. The Amphibian Research and Monitoring Initiative (ARMI): 5-Year Report. U.S. Geological Survey Scientific. Investigations Report 2006-5224.

Muthukkaruppan, Vr., P. Kanakambika, V. Manickavel, and K. Veeraraghavan. 1970. Analysis of the development of the lizard, *Calotes versicolor*. I. A series of normal stages in the embryonic development. Journal of Morphology 130:479–489.

Muul, I., and B. L. Lim. 1970. Vertical zonation in a tropical rain forest in Malaysia: Method of study. Science (USA) 169:788–789.

Myers, C. W. 1974. The systematics of *Rhadinaea* (Colubridae), a genus of New World snakes. Bulletin of the American Museum of Natural History 153:1–262.

Myers, R. L. 1990. Scrub and high pine. Pp. 150–193. In R. L. Myers and J. J. Ewel (eds.), *Ecosystems of Florida*. University of Central Florida Press, Orlando, Florida.

Naef-Daenzer, B., D. Früh, M. Stalder, P. Wetli, and E. Weise. 2005. Miniaturization (0.2·g) and evaluation of attachment techniques of telemetry transmitters. Journal of Experimental Biology 208:4063–4068.

Nagorsen, D. W., and L. R. Peterson. 1980. *Mammal Collector's Manual: A Guide for Collecting, Documenting, and Preparing Mammal Specimens for Scientific Research*. Life Sciences Miscellaneous Publications, Royal Ontario Museum, Toronto, Ontario, Canada.

Naulleau, G., and X. Bonnet. 1996. Body condition threshold for breeding in a viviparous snake. Oecologia 107:301–306.

Nečas, P. 2004. *Chameleons. Nature's hidden jewels*. Chimaira, Frankfurt am Main, Germany.

Neill, W. T. 1958. The occurrence of amphibians and reptiles in saltwater areas, and a bibliography. Bulletin of Marine Science of the Gulf and Caribbean 8:1–97.

Neill, W. T. 1964. Taxonomy, natural history, and zoogeography of the Rainbow Snake *Farancia erytrogramma* (Palisot de Beauvois). American Midland Naturalist 71:257–295.

Neill, W. T. 1965. Notes on aquatic snakes, *Natrix* and *Tretanorhinus,* in Cuba. Herpetologica 21:62–67.

Neill, W. T. 1971. *The Last of the Ruling Reptiles: Alligators, Crocodiles and Their Kin*. Columbia University Press, New York.

Nelson, D. H., and J. W. Gibbons. 1972. Ecology, abundance, and seasonal activity of the Scarlet Snake, *Cemophora coccinea*. Copeia 1972:582–584.

Newbold, T. A. S. 2005. Desert Horned Lizard (*Phrynosoma platyrhinos*) locomotor performance: The influence of Cheatgrass (*Bromus tectorum*). Southernwestern Naturalist 50:17–23.

Newman, D. G. (ed.). 1982. *New Zealand Herpetology*. Proceedings of a symposium held at Victoria University of Wellington 29–31 January 1980. Wildlife Service, Department of Internal Affairs, Wellington, New Zealand Occasional Publication No. 2.

Newman, D. G. 1987. *Tuatara*. McIndoe, Dunedin, New Zealand.

Nguyen, V. S., T. C. Ho, and Q. T. Nguyen. 2009. *Herpetofauna of Vietnam*. Chimaira, Frankfurt am Main, Germany.

Nichols, O. C., R. D. Kenney, and M. W. Brown. 2008. Spatial and temporal distribution of North Atlantic Right Whales (*Eubalaena glacialis*) in Cape Cod Bay, and implications for management. Fishery Bulletin 106:270–280.

Nickerson, M. A. 1970. New uses for an old method used in ophidian sex determination. British Journal of Herpetology 4:138–139.

Norman, D. R. 1987. Man and tegu lizards in eastern Paraguay. Biological Conservation 41:39–56.

Noro, M., A. Uejima, G. Abe, M. Manabe, and K. Tamura. 2009. Normal developmental stages of the Madagascar Ground Gecko *Paroedura pictus* with special reference to limb morphogenesis. Developmental Dynamics 238:100–109.

Norris, K. S. 1958. The evolution and systematics of the iguanid genus *Uma* and its relation to the evolution of other North American desert reptiles. Bulletin American Museum Natural History 114(3):247–326.

Northcutt R. G., and A. B. Butler. 1974. Retinal projections in the Northern Water Snake *Natrix sipedon sipedon* (L). Journal of Morphology 142:117–135.

Norton, T. M., J. Spratt, J. Behler, and K. Hernandez. 1998. Medetomidine and ketamine anesthesia with atipamizole reversal in private free-ranging Gopher Tortoises, *Gopherus polyphemus*. *Association of Reptilian and Amphibian Veterinarians Annual Proceedings* 1998:25–27.

Noss, A. J., I. Oetting, and R. L. Cuéllar. 2005. Hunter self-monitoring by the Isoseño-Guaraní in the Bolivian Chaco. Biodiversity and Conservation 14:2679–2693.

Noss, R. F. 1990. Indicators for monitoring biodiversity: A hierarchical approach. Conservation Biology 4:355–364.

Novaro, A. J., R. Bodmer, and K. H. Redford. 1999. Sustentabilidad de la caza en el Neotrópico: ¿Cuán comunes son los sistemas de fuente y sumidero? Pp. 27–31. In T. G. Fang, O. L. Montenegro, and R. E. Bodmer (eds.), *Manejo y Conservación de Fauna Silvestre en América Latina*. Editorial-Instituto de Ecología, La Paz, Bolivia.

Nuñez Otaño, N. B., A. Imhof, P. G. Bolcatto, and A. Larriera. 2010. Sex differences in the genitalia of hatchling *Caiman latirostris*. Herpetological Review 41:32–35.

Nussbaum, R. A., C. J. Raxworthy, A. P. Raselimanana, and J.-B. Ramanamanjato. 1999. Amphibians and reptiles of the Réserve Naturelle Intégrale d'Andohahela, Madagascar. Fieldiana Zoology n.s. 94:155–173.

Nutaphand, W. 1979. *The Turtles of Thailand*. Mitbhadung, Bangkok, Thailand.

O'Brien, T. G. 1990. A comparison of 3 survey methods for estimating relative abundance of rare crocodilians. Pp. 91–108. In *Croccodiles: Proceedings of the 10th Annual Working Meeting of the Crocodile Specialist Group of the Species Survival Commission of IUCN-The World Conservation Union*. Vol. 2. IUCN-The World Conservation Union, Gland, Switzerland.

Obst, F. J. 1988. *Turtles, Tortoises and Terrapins*. St. Martin's, New York.

O'Keefe, F. R. 2002. The evolution of plesiosaur and pliosaur morphotypes in the Plesiosauria (Reptilia: Sauropterygia). Paleobiology 28:101–112.

Oliver, I., and A. J. Beattie. 1993. A possible method for the rapid assessment of biodiversity. Conservation Biology 7:562–568.

Olson, S. L., and H. F. James. 1982. Fossil birds from the Hawaiian Islands: Evidence for wholesale extinction by man before Western contact. Science 217:633–635.

Orenstein, R. 2001. *Turtles, Tortoises and Terrapins: Survivors in Armor.* Firefly, Buffalo, New York.

Orlova, V. F., and D. V. Semenov. 1986. Distribution of amphibians and reptiles in Mongolia. Pp. 91–108. In *Zoogeographical regionalization of Mongolian People's Republic*. USSR Academy of Sciences, Moscow, Soviet Union. [in Russian].

Orós, J., J. L. Rodríguez, A. Espinosa de los Monteros, F. Rodríguez, P. Herráez, and A. Fernández. 1997. Tracheal malformation in a bicephalic Honduran milk snake (*Lampropeltis hondurensis*) and subsequent fatal *Salmonella arizonae* infection. Journal of Zoo and Wildlife Medicine 28:331–335.

O'Shea, M. 1996. *A Guide to the Snakes of Papua New Guinea*. Independent Publishing, Independent Group, Port Moresby, Papua New Guinea.

Otis, D. L., K. P. Burnham, G. C. White, and D. R. Anderson. 1978. Statistical inference from capture data on closed animal populations. Wildlife Monographs 62:1–135.

Overton, W. 1971. Estimating the number of animals in wildlife populations. Pp. 403–456. In R. H. Giles, Jr. (ed.), *Wildlife Management Techniques*. 3rd ed., rev. Wildlife Society, Washington, DC.

Owen, J. G., and J. R. Dixon. 1989. An ecogeographic analysis of the herpetofauna of Texas. Southwestern Naturalist 34:165–180.

Owens, A. K., K. R. Moseley, T. S. McCay, S. B. Castleberry, J. C. Kilgo, and W. M. Ford. 2008. Amphibian and reptile community response to coarse woody debris manipulations in upland Loblolly Pine (*Pinus taeda*) forests. Forest Ecology and Management 256:2078–2083.

Owens, D. W. 1995. The role of reproductive physiology in the conservation of sea turtles. Pp. 39–44. In K. A. Bjorndal (ed.), *Biology and Conservation of Sea Turtles*. Rev. ed. Smithsonian Institution Press, Washington, DC.

Owens, D. W. 1999. Reproductive cycles and endocrinology. Pp. 119–123. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, and M. Donnelly (eds.), *Research and Management Techniques for the Conservation of Sea Turtles*. IUCN/SSC Marine Turtle Specialist Group Publication no. 4.

Owens, D. W., and G. J. Ruiz. 1980. New methods of obtaining blood and cerebrospinal fluid from marine turtles. Herpetologica 36:17–20.

Paarmann, W., and K. Kerck. 1997. Advances in using the canopy fogging technique to collect living arthropods from tree-crowns. Pp. 53–66. In N. E. Stork, J. Adis, and R. K. Didham (eds.), *Canopy Arthropods*. Chapman Hall, London, England.

Pacheco, L. F. 1996. Wariness of caiman populations and its effect on abundance estimates. Journal of Herpetology 30:123–126.

Palka, D. 2000. Abundance and distribution of sea turtles estimated from data collected during cetacean surveys. Pp. 71–72. In K. A. Bjorndal and A. B. Bolten (eds.), *Proceedings of a Workshop on Assessing Abundance and Trends for In-Water Sea Turtle Populations*. NOAA Technical Memorandum NMFS-SEFSC-445. National Marine Fisheries Service, Miami, Florida.

Palmer, B. D., V. G. Demarco, and L. J. Guillette, Jr. 1993. Oviductal morphology and eggshell formation in the lizard, *Sceloporus woodi*. Journal of Morphology 217:205–217.

Palmer, M. W. 1990. The estimation of species richness by extrapolation. Ecology 71:1195–1198.

Parham, J. F., and G. R. Zug. 1997. Age and growth of Loggerhead Sea Turtles (*Caretta caretta*) of coastal Georgia: An assessment of skeletochronological age-estimates. Bulletin of Marine Science 61:287–304.

Parker, W. S. 1972. Aspects of the ecology of a Sonoran desert population of the Western Banded Gecko, *Coleonyx variegatus* (Sauria, Eublepharinae). American Midland Naturalist 88:209–224.

Parker, W. S., and W. S. Brown. 1973. Species composition and population changes in two complexes of snake hibernacula in northern Utah. Herpetologica 29:319–326.

Parker, W. S., and W. S. Brown. 1980. Comparative ecology of two colubrid snakes, *Masticophis t. taeniatus* and *Pituophis melanoleucus deserticola,* in northern Utah. Milwaukee Public Museum Publications in Biology and Geology 7:1–104.

Parker, W. S., and M. V. Plummer. 1987. Population ecology. Pp. 253–301. In R. A. Seigel, J. T. Collins, and S. S. Novak (eds.), *Snakes: Ecology and Evolutionary Biology*. McGraw-Hill, New York.

Parmelee, J. R., and H. S. Fitch. 1995. An experiment with artificial shelters for snakes: Effects of material, age, and surface preparation. Herpetological Natural History 3:187–191.

Parmenter, C. J. 1993. A preliminary evaluation of the performance of passive integrated transponders and metal tags in a population study of the Flatback Sea Turtle (*Natator depressus*). Wildlife Research 20:375–381.

Passek, K. M., and M. E. Collver. 2001. Technique for temporarily marking lizards that does not require capture. Herpetological Review 32:30–31.

Pasteels, J. J. 1970. Développement embryonnaire. Pp. 893–971. In P.-P. Grassé (ed.), *Traité de Zoologie, Anatomie, Systématique, Biologie: Reptiles*. Vol. 14, fasc. III. Masson, Paris, France.

Paulissen, M. A. 1986. A technique for marking teiid lizards in the field. Herpetological Review 17:16–17.

Pauwels, O. S. G., and J. P. Vande weghe. 2008. *Les Reptiles du Gabon*. Smithsonian Institution, Washington, DC.

Pauwels, O. S. G., V. Wallach, and P. David. 2008. Global diversity of snakes (Serpentes; Reptilia) in freshwater. Hydrobiologia 595:599–605.

Pearcy, R. W., J. Ehleringer, H. A. Mooney, and P. W. Rundel (eds.). 1989. *Plant Physiological Ecology: Field Methods and Instrumentation*. Chapman Hall, New York.

Pearse, A. G. E. 1980. *Histochemistry: Theoretical and Applied*. 3 vols. 4th ed. Churchill Livingstone, New York.

Pearson, D., R. Shine, and A. Williams. 2002. Geographic variation in sexual size dimorphism within a single snake species (*Morelia spilota*, Pythonidae). Oecologia 131:418–426.

Pearson, P. G. 1955. Population ecology of the Spadefoot Toad, *Scaphiopus h. holbrooki* (Harlan). Ecological Monographs 25:233–267.

Pechmann, J. H. K., D. E. Scott, R. D. Semlitsch, J. P. Caldwell, L. J. Vitt, and J. W. Gibbons. 1991. Declining amphibian populations: The problem of separating human impacts from natural fluctuations. Science 253:892–895.

Pechmann, J. H. K., and H. M. Wilbur.1994. Putting declining amphibian populations in perspective: Natural fluctuations and human impacts. Herpetologica 50:65–84.

Pedrono, M., and L. L. Smith. 2003. Testudinidae, land tortoises. Pp. 951–956. In S. M. Goodman and J. P. Benstead. (eds.), *The Natural History of Madagascar*. University of Chicago Press, Chicago, Illinois.

Peet, R. K. 1974. The measurement of species diversity. Annual Review of Ecology and Systematics 5:285–307.

Peet, R. K. 1975. Relative diversity indices. Ecology 56:496–498.

Penney, K. M., K. D. Gianopulos, E. D. McCoy, and H. R. Mushinsky. 2001. The visible implant elastomer marking technique in use for small reptiles. Herpetological Review 32:236–241.

Pérez-Santos, C., and A. G. Moreno. 1988. *Ofidios de Colombia*. Monografie VI. Museo Regionale di Scienze Naturali, Torino, Italy.

Pérez-Santos, C., and A. G. Moreno. 1991. *Ofidios de Ecuador*. Monografie XI. Museo Regionale di Scienze Naturali, Torino, Italy.

Perison D., J. Phelps, C. Pavel, and R. Kellison. 1997. The effects of timber harvest in a South Carolina blackwater bottomland. Forest Ecology and Management 90:171–185.

Peterman, R. M. 1990. Statistical power analysis can improve fisheries research and management. Canadian Journal of Fisheries and Aquatic Science 47:2–15.

Peterson, B. L., B. E. Kus, and D. H. Deutschman. 2004. Determining nest predators of the Least Bell's Vireo through point counts, tracking stations, and video photography. Journal of Field Ornithology 75:89–95.

Peterson, C. R. 1987. Daily variation in the body temperatures of free-ranging garter snakes. Ecology 68:160–169.

Peterson, C. R. 1997. Checklist for amphibian survey reports. Pp. 113–122. In D. H. Olson, W. P. Leonard, and R. B. Bury (eds.), *Sampling Amphibians in Lentic Habitats: Methods and Approaches for the Pacific Northwest*. Society for Northwestern Vertebrate Biology, Northwest Fauna no. 4.

Peterson, C. R., S. R. Burton, and D. A. Patla. 2005. Geographic Information Systems and Survey Designs. Pp. 320–325. In M. J. Lannoo (ed.), *Amphibian Declines: The Conservation Status of United States Species*. University of California Press, Berkeley, California.

Peterson, C. R., and M. E. Dorcas. 1992. The use of automated data-acquisition techniques in monitoring amphibian and reptile populations. Pp. 369–378. In D. R. McCullough and R. H. Barrett (eds.), *Wildlife 2001: Populations*. Elsevier Science, Barking, Essex, England.

Peterson, C. R., and M. E. Dorcas. 1994. Automated data acquisition. Pp. 47–57. In W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster (eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Smithsonian Institution Press, Washington, DC.

Peterson, C. R., A. R. Gibson, and M. E. Dorcas. 1993. Snake thermal ecology: The causes and consequences of body-temperature variation. Pp. 241–314. In R. A. Seigel and J. T. Collins (eds.), *Snakes: Ecology and Behavior.* McGraw-Hill, New York.

Pethiyagoda, R., and K. Manamendra-Arachchi. 1998. Evaluating Sri Lanka's amphibian diversity. Occasional Papers of the Wildlife Heritage Trust [Sri Lanka] 2:1–12.

Petokas, P. J., and M. M. Alexander. 1979. A new trap for basking turtles. Herpetological Review 10:90.

Petranka, J. W., M. E. Eldridge, and K. E Haley. 1993. Effects of timber harvesting on southern Appalachian salamanders. Conservation Biology 7:363–370.

Pianka, E. R. 1986. *Ecology and Natural History of Desert Lizards: Analysis of the Ecological Niche and Community Structure*. Princeton University Press, Princeton, New Jersey.

Pianka, E. R., and L. J. Vitt. 2003. *Lizards: Windows to the Evolution of Diversity.* University of California Press, Berkeley, California.

Pielou, E. C. 1969. *An Introduction to Mathematical Ecology.* John Wiley, New York.

Pielou, E. C. 1975. *Ecological Diversity*. John Wiley, New York.

Pielou, E. C. 1977. *Mathematical Ecology.* Wiley, New York.

Pietruszka, R. D. 1981. An evaluation of stomach flushing for desert lizard diet analysis. Southwestern Naturalist 26:101–105.

Pike, D. A., K. S. Peterman, and J. H. Exum. 2007. Use of altered habitats by the endemic Sand Skink (*Plestiodon reynoldsi* Stejneger). Southeastern Naturalist 6:715–726.

Pike, D. A., K. S. Peterman, and J. H. Exum. 2008. Habitat structure influences the presence of Sand Skinks (*Plestiodon reynoldsi*) in altered habitats. Wildlife Research 35:120–127.

Pike, D. A., K. S. Peterman, R. S. Mejeur, M. D. Green, K. D. Nelson, and J. H. Exum. 2008. Sampling techniques and methods for determining the spatial distribution of Sand Skinks (*Plestiodon reynoldsi*). Florida Scientist 71:93–104.

Pilliod, D. S., and C. R. Peterson. 2000. Evaluating effects of fish stocking on amphibian populations in wilderness lakes. Pp. 328–335. In D. N. Cole, S. F. McCool, W. T. Borrie, and J. O'Loughlin (compilers). *Proceedings: Wilderness Science in a Time of Change,* Vol. 5. *Wilderness Ecosystems, Threats, and Management*. RMRS-P-15-VOL-5. U.S. Department of Agriculture, Forest Service, Rock Mountain Research Station, Ogden, Utah.

Pilorge, T., and J. Castanet. 1981. Détermination de l'âge dans une population naturelle du lézard vivipare (*Lacerta vivipara* Jacquin 1787). Acta Oecologia/Oecolgia Generalis 2:3–16.

Pimm, S. L., and A. Redfearn. 1988. The variability of population densities. Nature 334:613–614.

Pisani, G. R. 1973. A *Guide to Preservation Techniques for Reptiles and Amphibians*. Society for the Study of Amphibians and Reptiles, Herpetological Circular no. 1.

Pitman, C. R. S. 1941. About crocodiles. Uganda Journal 9:89–114.

Platt, D. R. 1969. Natural history of the hognose snakes *Heterodon platyrhinos* and *Heterodon nasicus*. University of Kansas Publications, Museum of Natural History 18:253–420.

Platt, S. G., Kalyar, and Win Ko Ko. 2000. Exploitation and conservation status of tortoises and freshwater turtles in Myanmar. Pp. 95–100. In P. P. van Dijk, B. L. Stuart, and A. G. J. Rhodin (eds.), *Asian Turtle Trade: Proceedings of a Workshop on Conservation and Trade of Freshwater Turtles and Tortoises in Asia, Phnom Penh, Cambodia, 1–4 December 1999*. Chelonian Research Monographs 2.

Platz, C. C., Jr., G. Mengden, H. Quin, F. Wood, and J. Wood. 1980. Semen collection, evaluation and freezing in the Green Sea Turtle, Galapagos Tortoise, and Red-eared Pond Turtle. *American Association of Zoo Veterinarians Annual Proceedings* 1980:47–54.

Pledger, S. 2005. The performance of mixture models in heterogeneous closed population capture-recapture. Biometrics 61:868–873.

Pledger, S., and M. Efford. 1998. Correction of bias due to heterogeneous capture probability in capture-recapture studies of open populations. Biometrics 54:888–898.

Pledger, S., K. H. Pollock, and J. L. Norris. 2003. Open capture-recapture models with heterogeneity: I. Cormack-Jolly-Seber model. Biometrics 59:786–794.

Plummer, M. V. 1977a. Activity, habitat and population structure in the turtle, *Trionyx muticus*. Copeia 1977:431–440.

Plummer, M. V. 1977b. Reproduction and growth in the turtle *Trionyx muticus*. Copeia 1977:440–447.

Plummer, M. V. 1979. Collecting and marking. Pp. 45–61. In M. Harless and H. Morlock (eds.), *Turtles: Perspectives and Research.* John Wiley, New York.

Plummer, M. V. 1980. Ventral scute anomalies in a population of *Opheodrys aestivus*. Journal of Herpetology 14:199.

Plummer, M. V. 1981. Habitat utilization, diet and movements of a temperate arboreal snake (*Opheodrys aestivus*). Journal of Herpetology 15:425–432.

Plummer, M. V. 1997. Population ecology of green snakes (*Opheodrys aestivus*) revisited. Herpetological Monographs 11:102–123.

Plummer, M. V. 2002. Observations on hibernacula and overwintering ecology of Eastern Hog-nosed Snakes (*Heterodon platirhinos*). Herpetological Review 33:89–90.

Plummer, M. V. 2003. Activity and thermal ecology of the box turtle, *Terrapene ornata,* at its southwestern range limit in Arizona. Chelonian Conservation and Biology 4:569–577.

Plummer, M. V. 2008. A notching system for marking softshell turtles. Herpetological Review 39:64–65.

Plummer, M. V., and J. C. Burnley. 1997. Behavior, hibernacula, and thermal relations of softshell turtles (*Trionyx spiniferus*) overwintering in a small stream. Chelonian Conservation and Biology 2:489–493.

Plummer, M. V., N. E. Mills, and S. L. Allen 1997. Activity, habitat, and movement patterns of softshell turtles (*Trionyx spiniferus*) in a small stream. Chelonian Conservation and Biology 2:514–520.

Pope, C. H. 1935. *The Reptiles of China: Turtles, Crocodilians, Snakes, Lizards*. American Museum of Natural History, New York.

Pough, F. H. 1991. *Recommendations for the Care of Amphibians and Reptiles in Academic Institutions*. ILAR News 33(4):S1–S21.

Pough, F. H., R. M. Andrews, J. E. Cadle, M. L. Crump, A. H. Savitzky, and K. D. Wells. 2003. *Herpetology.* 3rd ed. Prentice Hall, Upper Saddle River, New Jersey.

Pough, H. 1966. Ecological relationships of rattlesnakes in southeastern Arizona with notes on other species. Copeia 1966:676–683.

Powell, R., and R. W. Henderson (eds.). 1996. *Contributions to West Indian Herpetology. A Tribute to Albert Schwartz*. Contributions to

Herpetology Vol. 12. Society for the Study of Amphibians and Reptiles, Ithaca, New York.

Prendini, L., R. Hanner, and R. DeSalle. 2002. Obtaining, storing and archiving specimens and tissue samples for use in molecular studies. Pp. 176–248. In R. DeSalle, G. Giribet, and W. Wheeler (eds.), *Techniques in Molecular Systematics and Evolution*. Birkhäuser, Basel, Switzerland.

Prentice, E. F., T. A. Flagg, C. S. McCutcheon, and D. F. Brastow. 1990. PIT-tag monitoring systems for hydroelectric dams and fish hatcheries. Pp. 323–334. In N. C. Parker, A. E. Giorgi, R. C. Heidinger, and D. Jester (eds.), *Fish-marking Techniques*. American Fisheries Society Symposium no. 7. American Fisheries Society, Bethesda, Maryland.

Presnell, J. K., and M. P. Schreibman. 1997. *Humason's Animal Tissue Techniques*. 5th ed. Johns Hopkins University Press, Baltimore, Maryland.

Price, A. H. 1985. Roadriding As a Herpetofaunal Collecting Technique and Its Impact upon the Herpetofauna of New Mexico. Unpubl. Final report to the Endangered Species Program, New Mexico Department of Game and Fish, Contract no. 519-72-07, Albuquerque, New Mexico.

Price, A. H., and J. L. LaPointe. 1990. Activity patterns of a Chihuahuan desert snake community. Annals of Carnegie Museum 59:15–23.

Prior, K. A., and P. J. Weatherhead. 1996. Habitat features of Black Rat Snake hibernacula in Ontario. Journal of Herpetology 30:211–218.

Pritchard, M. H., and G. O. W. Kruse (compiler). 1982. *The Collection and Preservation of Animal Parasites*. University of Nebraska Press, Lincoln, Nebraska.

Pritchard, P. C. H. 1982. Nesting of the Leatherback Turtle, *Dermochelys coriacea* in Pacific Mexico, with a new estimate of the world population status. Copeia 1982:741–747.

Pritchard, P. C. H., and J. A. Mortimer. 1999. Taxonomy, external morphology, and species identification. Pp. 21–38. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, and M. Donnelly (eds.), *Research and Management Techniques for the Conservation of Sea Turtles*. IUCN/SSC Marine Turtle Specialist Group Publication no. 4.

Pritchard, P. C. H., and P. Trebbau. 1984. *The Turtles of Venezuela*. Contributions to Herpetology Vol. 2. Society for the Study of Amphibians and Reptiles, Oxford, Ohio.

Punay, E. Y. 1975. Commercial sea snake fisheries in the Philippines. Pp. 489–502. In W. A. Dunson (ed.), *The Biology of Sea Snakes*. University Park Press, Baltimore, Maryland.

Purcell, K. L. 1997. Use of a fiberscope for examining cavity nests. Journal of Field Ornithology 68:283–286.

Pye, G. W., and J. W. Carpenter. 1998. Ketamine sedation followed by propofol anesthesia in a slider, *Trachemy scripta,* to facilitate removal of an esophageal foreign body. Bulletin of the Association of Reptilian and Amphibian Veterinarians 8:16–17.

Quinn, H., and J. P. Jones. 1974. Squeeze box technique for measuring snakes. Herpetological Review 5:35.

Quinn, V. S., M. Klukosky, and D. K. Hews. 2001. Longevity of paint marks and the lack of effect on survivorship and growth of *Sceloporus* lizards. Herpetological Review 32:92–93.

Quirt, K. C., G. Blouin-Demers, B. J. Howes, and S. C. Lougheed. 2006. Microhabitat selection of Five-Lined Skinks in northern peripheral populations. Journal of Herpetology 40:335–342.

Raaijmakers, J. G. W. 1987. Statistical analysis of the Michaelis-Menten equation. Biometrics 43:793–803.

Raff, R. A. 1996. *The Shape of Life: Genes, Development, and the Evolution of Animal Form*. University of Chicago Press, Chicago, Illinois.

Rainey, W. E. 1981. *Guide to Sea Turtle Visceral Anatomy*. NOAA Technical Memorandum NMFS-SEFSC-443. National Marine Fisheries Service, Miami, Florida. http://www.aoml.noaa.gov/general/lib/turtles.htm

Rainwater, T. R., S. T. McMurry, and S. G. Platt. 1999. Ectromelia in Morelet's Crocodile from Belize. Journal of Wildlife Diseases 35:125–129.

Rall, A. E. 2004. Effects of Longleaf Pine Management Practices on the Herpetofauna of South Alabama. Unpubl. Master's thesis, Auburn University, Auburn, Alabama.

Ramírez-Bautista, A., and M. Benabib. 2001. Perch height of the arboreal lizard *Anolis nebulosus* (Sauria: Polychrotidae) from a tropical dry forest of México: Effect of the reproductive season. Copeia 2001:187–193.

Ramírez-Pinilla, M., V. H. Serrano, and J. C. Galeano. 2002. Annual reproductive activity of *Mabuya mabouya* (Squamata, Scincidae). Journal of Herpetology 36:667–677.

Rand, A. S., and C. W. Myers. 1990. The herpetofauna of Barro Colorado Island, Panama: An ecological summary. Pp 386–409. In A. H. Gentry (ed.), *Four Neotropical Rainforests*. Yale University Press, New Haven, Connecticut.

Rao, M. V. S., and B. S. Rajabai. 1972. Ecological aspects of the agamid lizards *Sitana ponticeriana* and *Calotes nemoricola* in India. Herpetologica 28:285–289.

Raselimanana, A. P., C. J. Raxworthy, and R. A. Nussbaum. 2000. Herpetofaunal species diversity and elevational distribution within the Parc National de Marojejy, Madagascar. Fieldiana Zoology n.s. 97:157–174.

Rashid, S. M. A., and C. H. Diong. 1999. Observations on *Varanus salvator* feeding on *Oligodon octolineatus*. Hamadryad 24:48–49.

Rasmussen, P. W., D. M. Heisey, E. V. Nordheim, and T. M. Frost. 1993. Time-series intervention analysis: Unreplicated large-scale experiments. Pp. 138–158. In S. M. Scheiner and J. Gurevitch (eds.), *Design and Analysis of Ecological Experiments*. Chapman Hall, New York.

Rasskin-Gutman, D., and A. D. Buscalioni. 2001. Theoretical morphology of the Archosaur (Reptilia: Diapsida) pelvic girdle. Paleobiology 27:59–78.

Raxworthy, C. J. 1988. Reptiles, rainforests and conservation in Madagascar. Biological Conservation 43:181–211.

Raxworthy, C. J., E. Martinez-Meyer, N. Horning, R. A. Nussbaum, G. E. Schneider, M. A. Ortega-Huerta, and A. T. Peterson. 2003. Predicting distributions of known and unknown reptiles species in Madagascar. Nature 426:837–841.

Raxworthy, C. J., and R. A. Nussbaum. 1994. A rainforest survey of amphibians, reptiles and small mammals at Montagne d'Ambre, Madagascar. Biological Conservation 69:65–73.

Raxworthy, C. J. and R. A. Nussbaum. 1996. Amphibians and reptiles of the Réserve Natural Intégrale d'Andringitra, Madagascar: A study of elevational distribution and local endemicity. Fieldiana Zoology n.s. 85:158–170.

Raynaud, A. 1961. Quelques phases du développement des œufs chez l'orvet (*Anguis fragilis* L.). Bulletin Biologique de la France et de la Belgique 95:365–384.

Reading, C. J. 1997. A proposed standard method for surveying reptiles on dry lowland heath. Journal of Applied Ecology 34:1057–1069.

Reagan, D. P. 1974. Habitat selection in the Three-toed Box Turtle, *Terrapene carolina triunguis*. Copeia 1974:512–527.

Reagan, D. P. 1992. Congeneric species distribution and abundance in a three-dimensional habitat: The rain forest anoles of Puerto Rico. Copeia 1992:392–403.

Reagan, D. P. 1995. Lizard ecology in the canopy of an island rain forest. Pp. 149–164. In M. D. Lowman and N. M. Nadkarni (eds.), *Forest Canopies*. Academic Press, San Diego, California.

Ream, C., and R. Ream. 1966. The influence of sampling methods on the estimation of population structure in Painted Turtles. American Midland Naturalist 75:325–338.

Reed, J. M. 1996. Using statistical probability to increase confidence of inferring species extinction. Conservation Biology 10:1283–1285.

Reed, J. M., and A. R. Blaustein. 1995. Assessment of "nondeclining" amphibian populations using power analysis. Conservation Biology 9:1299–1300.

Reed, R. N. 2001. Effects of museum preservation techniques on length and mass of snakes. Amphibia-Reptilia 22:488–491.

Reed R. N., J. M. Morton, and G. E. Desy. 2000. Use of monofiliment snare traps for capture of varanid lizards. Micronesica 33:99–104.

Regan, T. 1983. *The Case for Animal Rights*. University of California Press, Berkeley, California.

Regaud, C., and A. Policard. 1903. Recherches sur la structure du rein de quelques ophidiens. Archives d'Anatomie Microscopique 6:191–282 + plates VII–X.

Reichenbach-Klinke, H., and E. Elkan. 1965. *The Principal Diseases of Lower Vertebrates*. Academic Press, New York.

Reinert, H. K. 1984. Habitat variation within sympatric snake populations. Ecology 65:1673–1682.

Reinert, H. K. 1992. Radiotelemetric field studies of pitvipers: Data acquisition and analysis. Pp. 185–197. In J. A. Campbell and E. D. Brodie, Jr. (eds.), *The Biology of Pitvipers.* Selva, Tyler, Texas.

Reinert, H. K. 1993. Habitat selection in snakes. Pp. 201–240. In R. A. Seigel and J. T. Collins (eds.), *Snakes: Ecology and Behavior.* McGraw-Hill, New York.

Reinert, H. K., and D. Cundall. 1982. An improved surgical implantation method for radio-tracking snakes. Copeia 1982:702–705.

Reinert, H. K., and R. T. Zappalorti. 1988a. Timber Tattlesnakes (*Crotalus horridus*) of the Pine Barrens: Their movement patterns and habitat preference. Copeia 1988:964–978.

Reinert, H. K., and R. T. Zappalorti. 1988b. Field observation of the association of adult and neonatal timber rattlesnakes, *Crotalus horridus,* with possible evidence for conspecific trailing. Copeia 1988:1057–1059.

Renaud, M. L. 1995. Movements and submergence patterns of Kemp's Ridley Turtles (*Lepidochelys kempii*). Journal of Herpetology 29:370–374.

Renaud, M. L., G. R. Gitschlag, and J. K. Hale. 1993. Retention of imitation satellite transmitters fiberglassed to the carapace of sea turtles. Herpetological Review 24:94–99.

Renfrew, R. B., and C. A. Ribic. 2003. Grassland passerine nest predators near pasture edges identified on videotape. Auk 120:371–383.

Renken, R. B., W. K. Gram, D. K. Fantz, S. C. Richter, T. J. Miller, K. B. Ricke, B. Russell, and X. Wang. 2004. Effects of forest management on amphibians and reptiles in Missouri Ozark forests. Conservation Biology 18:174–188.

Renyi, A. 1961. On measures of entropy and information. Pp. 547–561. In J. Neyman (ed.), *Proceedings of the Fourth Berkeley Symposium on Mathematical Statistics and Probability*. Vol. 1. *Contributions to the Theory of Statistics*. University of California Press, Berkeley, California.

Rexstad, E., and K. P. Burnham. 1991. *User's Guide for Interactive Program CAPTURE.* Colorado Cooperative Fish & Wildlife Research Unit, Colorado State University, Fort Collins, Colorado.

Reynolds, R. P. 1982. Seasonal incidence of snakes in northeastern Chihuahua, Mexico. Southwestern Naturalist 27:161–166.

Rhodin, A. G. J., and R. A. Mittermeier.1976. *Chelodina parkeri,* a new species of chelid turtle from New Guinea, with a discussion of *Chelodina siebenrocki* Werner, 1901. Bulletin of the Museum of Comparative Zoology 147:465–488.

Rhodin, A. G. J., P. P. van Dijk, and J. F. Parham. 2008. Turtles of the world: Annotated checklist of taxonomy and synonymy. Pp. 1–38. In A. G. J. Rhodin, P. C. H. Pritchard, P. P. van Dijk, R. A. Saumure, K. A. Buhlmann, and J. B. Iverson (eds.), *Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group.* Chelonia Research Monographs No. 5.

Ribeiro-Júnior, M. A., T. A. Gardner, and T. C. S. Ávila-Pires. 2006. The effectiveness of glue traps to sample lizards in a tropical rainforest. South American Journal of Herpetology 1:131–137.

Rice, A. N., J. P. Ross, A. G. Finger, and R. Owen. 2005. Application and evaluation of a stomach flushing technique for alligators. Herpetological Review 36:400–401.

Rice, C. G., E. E. Jorgensen, and S. Demarais. 1994. A comparison of herpetofauna detection and capture techniques in southern New Mexico. Texas Journal of Agriculture and Natural Resources 7:107–114.

Richards, J. A., and X. Jia. 1999. *Remote Sensing Digital Image Analysis: An Introduction.* 3rd ed. Springer, Berlin, Germany.

Richardson, D. M., J. W. Bradford, P. G. Range, and J. Christensen. 1999. A video probe system to inspect Red-cockaded Woodpecker cavities. Wildlife Society Bulletin 27:353–356.

Richardson, K. C., G. J. W. Webb, and S. C. Manolis. 2002. *Crocodiles: Inside Out. A Guide to the Crocodilians and Their Functional Morphology.* Surrey Beatty, Chipping Norton, New South Wales, Australia.

Richmond, J. Q. 1998. Backpacks for lizards: A method for attaching radio transmitters. Herpetological Review 29:220–221.

Richter, K. O., and A. L. Azous. 1995. Amphibian occurrence and wetland characteristics in the Puget Sound Basin. Wetlands 15:305–312.

Rieppel, O. 1993. Patterns of diversity in the reptilian skull. Pp. 344–390. In J. Hanken and B. K. Hall (eds.), *The Skull*. Vol. 2. *Patterns of Structural and Systematic Diversity.* University of Chicago Press, Chicago, Illinois.

Riley, J., and F. W. Huchzermeyer. 1999. African Dwarf Crocodiles in the Likouala Swamp forests of the Congo Basin: Habitat, density, and nesting. Copeia 1999:313–320.

Rivas, J. A. 2000. The Life History of the Green Anaconda (*Eunectes murinus*) with Emphasis on Its Reproductive Biology. Unpubl. Ph.D. dissert., University of Tennessee, Knoxville, Tennessee.

Rivas, J. A., R. E. Ascanio, and M. D C. Muñoz. 2008. What is the length of a snake? Contemporary Herpetology 2008(2):1–3.

Rivas, J. A., and T. M. Ávila. 1996. Sex identification in juvenile Green Iguanas (*Iguana iguana*) by cloacal analysis. Copeia 1996:219–221.

Rivas, J. A., and G. M. Burghardt. 2002. Crotalomorphism: A metaphor to understand anthropomorphism by omission. Pp. 9–17. In M. Bekoff, C. Allen, and G. M. Burghardt (eds.), *The Cognitive Animal: Empirical and Theoretical Perspectives on Animal Cognition.* MIT Press, Cambridge, Massachusetts.

Rivas, J. A., C. R. Molina, and T. M. Ávila. 1996. A non-flushing stomach wash technique for large lizards. Herpetological Review 27:72–73.

Rivas, J. A., M. D. C. Muñoz, J. Thorbjarnarson, W. Holmstrom, and P. Calle. 1995. A safe method for handling large snakes in the field. Herpetological Review 26:138–139.

Roark, A. W., and M. E. Dorcas. 2000. Regional body temperature variation in corn snakes measured using temperature-sensitive passive integrated transponders. Journal of Herpetology 34:481–485.

Robb, J. 1977. *The Tuatara*. Meadowfield, Durham, England.

Robb, J. 1986. *New Zealand Amphibians and Reptiles in colour.* Collins, Auckland, New Zealand.

Robert, K. A., and M. B. Thompson. 2003. Reconstructing thermochron ibuttons to reduce size and weight as a new technique in the study of small animal thermal biology. Herpetological Review 34:130–132.

Roberts, E. D., C. L. Matlock, T. Joanen, L. McNease, and M. Bowen. 1988. Bone morphometrics and tetracycline marking patterns in young growing American Alligators (*Alligator mississippiensis*). Journal of Wildlife Disease 24:67–70.

Robinson, K. M., and G. G. Murphy. 1975. A new method for trapping softshell turtles. Herpetological Review 6:111.

Rocha, C. F. D., M. Van Sluys, M. A. S. Alves, H. G. Bergallo, and D. Vrcibradic. 2000. Activity of leaf-litter frogs: When should frogs be sampled? Journal of Herpetology 34:285–287.

Rocha, C. F. D., M. Van Sluys, M. A. S. Alves, H. G. Bergallo, and D. Vrcibradic. 2001. Estimates of forest floor litter frog communities: A comparison of two methods. Austral Ecology 26:14–21.

Rodda, G. H. 1992. Foraging behaviour of the Brown Tree Snake, *Boiga irregularis*. Herpetological Journal 2:110–114.

Rodda, G. 1993. Where's Waldo (and the snakes)? Herpetological Review 24:44–45.

Rodda, G. H., B. C. Bock, G. M. Burghardt, and A. S. Rand. 1988. Techniques for identifying individual lizards at a distance reveal influences of handling. Copeia 1988:905–913.

Rodda, G. H., and E. W. Campbell. 2002. Distance sampling of forest snakes and lizards. Herpetological Review 33:271–274.

Rodda, G. H., E. W. Campbell, III, and T. H. Fritts. 2001. A high validity census technique for herpetofaunal assemblages. Herpetological Review 32:24–30.

Rodda, G. H., E. W. Campbell, III, T. H. Fritts, and C. S. Clark. 2005. The predictive power of visual searching. Herpetological Review 36:259–264.

Rodda, G. H., K. Dean-Bradley, and T. H. Fritts. 2005. Glueboards for estimating lizard abundance. Herpetological Review 36:252–259.

Rodda, G. H., and T. H. Fritts. 1992. Sampling techniques for an arboreal snake, *Boiga irregularis*. Micronesica 25:23–40.

Rodda, G. H., T. H. Fritts, and E. W. Campbell, III. 1999. The feasibility of controlling the Brown Treesnake in small plots. Pp. 468–477. In G. H. Rodda, Y. Sawai, D. Chiszar, and H. Tanaka (eds.), *Problem Snake Management: The Habu and the Brown Treesnake*. Comstock, Cornell University Press, Ithaca, New York.

Rodda, G. H., T. H. Fritts, E. W. Campbell III, K. Dean-Bradley, G. Perry, and C. P. Qualls. 2002. Practical concerns in the eradication of island snakes. Pp. 260–265. In C. R. Veitch and M. N. Clout (eds.), *Turning the Tide: The Eradication of Invasive Species. Proceedings of the International Conference on Eradication of Island*

Invasives. IUCN SSC Invasive Species Specialist Group, IUCN, Gland, Switzerland. Occasional Paper of the IUCN Species Survival Commission no. 27.

Rodda, G. H., T. H. Fritts, C. S. Clark, S. W. Gotte, and D. Chiszar. 1999. A state-of-the-art trap for the Brown Treesnake. Pp. 268–305. In G. H. Rodda, Y. Sawai, D. Chiszar, and H. Tanaka (eds.), *Problem Snake Management: The Habu and the Brown Treesnake*. Comstock, Cornell University Press, Ithaca, New York.

Rodda, G. H., T. H. Fritts, M. J. McCoid, and E. W. Campbell, III. 1999. An overview of the biology of the Brown Treesnake (*Boiga irregularis*) a costly introduced pest on Pacific Islands. Pp. 44–80. In G. H. Rodda, Y. Sawai, D. Chiszar, and H. Tanaka (eds.), *Problem Snake Management: The Habu and the Brown Treesnake*. Comstock, Cornell University Press, Ithaca, New York.

Rodda, G. H., M. J. McCoid, and T. H. Fritts. 1993. Adhesive trapping II. Herpetological Review 24:99–100.

Rodda, G. H., and M. Nishimura. 1999. The effectiveness of snake traps worldwide. Pp. 285–305. In G. H. Rodda, Y. Sawai, D. Chiszar, and H. Tanaka (eds.), *Problem Snake Management: The Habu and the Brown Treesnake*. Comstock, Cornell University Press, Ithaca, New York.

Rodda, G. H., G. Perry, R. J. Rondeau, and J. Lazell. 2001. The densest terrestrial vertebrate. Journal of Tropical Ecology 17:331–338.

Rogers, A. R. 2001. Recent telemetry technology. Pp. 79–121. In J. J. Millspaugh and J. M. Marzluff (eds.), *Radio Tracking and Animal Populations*. Academic Press, San Diego, California.

Rollin, B. E. 1989. *The Unheeded Cry: Animal Consciousness, Animal Pain, and Science*. Oxford University Press, Oxford, England.

Romer, A. S. 1956. *Osteology of the Reptiles*. University of Chicago Press, Chicago, Illinois.

Romero L. M., and M. Wikelski. 2002. Exposure to tourism reduces stress-induced corticosterone levels in Galápagos marine iguanas. Biological Conservation 108:371–374.

Romero-Schmidt, H., A. Ortega-Rubio, C. Arguelles-Méndez, R. Coria-Benet, and F. Solis-Márin. 1994. The effect of two years of livestock grazing exclosure upon abundance in a lizard community in Baja California Sur, Mexico. Bulletin of the Chicago Herpetological Society 29:245–248.

Rooney, M. B., G. Levine, J. Gaynor, E. Macdonald, and J. Wimsatt. 1999. Sevoflurane anesthesia in Desert Tortoises (*Gopherus agassizii*). Journal of Zoo and Wildlife Medicine 30:64–69.

Rootes, W. L., and R. H. Chabreck. 1993a. Cannibalism in the American Alligator. Herpetologica 49:99–107.

Rootes, W. L., and R. H. Chabreck. 1993b. Reproductive status and movement of adult female alligators. Journal of Herpetology 27:121–126.

Rosen, P. C., and C. H. Lowe. 1994. Highway mortality of snakes in the Sonoran Desert of southern Arizona. Biological Conservation 68:143–148.

Rose, F. L., and D. Armentrout. 1974. Population estimates of *Ambystoma tigrinum* inhabiting two playa lakes. Journal of Animal Ecology 43:671–679.

Rosenzweig, M. L. 1995. *Species Diversity in Space and Time*. Cambridge University Press, Cambridge, England.

Ross, C. A. (ed.). 1989. *Crocodiles and Alligators*. Facts on File, New York.

Ross, J. P. 1998. Estimations of the nesting population size of Loggerhead Sea Turtles, *Caretta caretta*, Masirah Island, Sultanate of Oman. Pp. 90–93. In S. P. Epperly and J. Braun (compilers), *Proceedings of the Seventeenth Annual Sea Turtle Symposium*. NOAA Technical Memorandum NMFS-SEFSC-415. National Marine Fisheries Service, Miami, Florida.

Rossell, C. R., Jr., and J. L. Sheehan. 1998. Comparison of histological staining procedures for skeletochronological studies. Herpetological Review 29:95.

Rossi, J. V., and J. J. Feldner. 1993. *Crotalus willardi willardi* (Arizona Ridgenose Rattlesnake) and *Crotalus lepidus klauberi* (Banded Rock Rattlesnake). Arboreal behavior. Herpetological Review 24:35.

Rossman, D. A., N. B. Ford, and R. A. Seigel. 1996. *The Garter Snakes: Evolution and Ecology*. University of Oklahoma Press, Norman, Oklahoma.

Rostal, D. C., J. S. Grumbles, V. A. Lance, and J. R. Spotila. 1994. Non-lethal sexing techniques for hatchling and immature Desert Tortoises (*Gopherus agassizii*). Herpetological Monographs 8:83–87.

Roughton, R. D., and M. W. Sweeny. 1982. Refinements in scent-station methodology for assessing trends in carnivore populations. Journal of Wildlife Management 46:217–229.

Row, J. R., G. Blouin-Demers, and P. J. Weatherhead. 2007. Demographic effects of road mortality in Black Ratsnakes (*Elaphe obsoleta*). Biological Conservation 137:117–124.

Rowe, J. W. 1992. Dietary habits of the Blanding's Turtle (*Emydoidea blandingi*) in northeastern Illinois. Journal of Herpetology 26:111–114.

Royle, J. A. 2004a. Modeling abundance index data from anuran calling surveys. Conservation Biology 18:1378–1385.

Royle, J. A. 2004b. *N*-mixture models for estimating population size from spatially replicated counts. Biometrics 60:108–115.

Royle, J. A., and J. D. Nichols. 2003. Estimating abundance from repeated presence-absence data or point counts. Ecology 84:777–790.

Roze, J. A. 1996. *Coral snakes of the Americas: Biology, Identification, and Venoms*. Krieger, Malabar, Florida.

Rudolph, D. C., and S. J. Burgdorf. 1997. Timber Rattlesnakes and Louisiana Pine Snakes of the West Gulf Coastal Plain: Hypotheses of decline. Texas Journal of Science 49(3) Supplement:111–122.

Rudolph, D. C., S. J. Burgdorf, R. N. Conner, and R. R. Schaefer. 1999. Preliminary evaluation of the impact of roads and associated vehicular traffic on snake populations in eastern Texas. Pp. 129–136. In G. L. Evink, P. Garrett, and D. Zeigler (eds.), *Proceedings of the Third International Conference on Wildlife Ecology and Transportation: September 13–16, 1999, Missoula, Montana*. FL-ER-73-99. Florida Department of Transportation, Tallahassee, Florida.

Rudolph, D. C., S. J. Burgdorf, R. R. Schaefer, R. N. Conner, and R. T. Zappalorti. 1998. Snake mortality associated with late season radio-transmitter implantation. Herpetological Review 29:155–156.

Rudolph, D. C., and J. G. Dickson. 1990. Streamside zone width and amphibian and reptile abundance. Southwest Naturalist 35:472–476.

Rueda-Almonacid, J. V., J. L. Carr, R. A. Mittermeier, J. V. Rodríguez-Mahecha, R. B. Mast, R. C. Vogt, A. G. J. Rhodin, J. de la Ossa-Velásquez, J. N. Rueda, and C. G. Mittermeier. 2007. *Las Tortugas y los Cocodrilianos de los Países Andinos del Trópico*. Serie de Guías Tropicales de Campo No. 6. Conservación Internacional, Bogotá, Colombia.

Ruedas, L. A., J. Salazar-Bravo, J. W. Dragoo, and T. L. Yates. 2000. The importance of being earnest: What, if anything, constitutes a "specimen examined?" Molecular Phylogenetics and Evolution 17:129–132.

Russell, K. R., D. C. Guynn, Jr., and H. G. Hanlin. 2002. Importance of small isolated wetlands for herpetofaunal diversity in managed, young growth forests in the coastal plain of South Carolina. Forest Ecology and Management 163:43–49.

Russell, K. R., and H. G. Hanlin. 1999. Aspects of the ecology of worm snakes (*Carphophis amoenus*) associated with small isolated wetlands in South Carolina. Journal of Herpetology 33:339–344.

Russell, K. R., H. G. Hanlin, T. B. Wigley, and D. C. Guynn, Jr. 2002. Responses of isolated wetland herpetofauna to upland forest management. Journal of Wildlife Management 66:603–617.

Russell, W. M. S., and R. L. Burch. 1959. *The Principles of Humane Experimental Technique*. Methuen, London, England.

Russo, L.-M. 1990. Animals in science: The justification issue. Pp. S8–S13. In S. Donnelley and K. Nolan (eds.), *Animals, Science, and Ethics*. Hastings Center Report Vol. 20(3) Special Supplement.

Ruthven III, D. C., R. T. Kazmaier, and M. W. Janis. 2008. Short-term response of herpetofauna to various burning regimes in the south Texas plains. Southwestern Naturalist 53:480–487.

Ryan, T. J., T. Philippi, Y. A. Leiden, M. E. Dorcas, T. B. Wigley, and J. W. Gibbons. 2002. Monitoring herpetofauna in a managed forest landscape: Effects of habitat types and census techniques. Forest Ecology and Management 167:83–90.

Sabo, J. L. 2003. Hot rocks or no hot rocks: Overnight retreat availability and selection by a diurnal lizard. Oecologia 136:329–335.

Sacchi, R., S. Scali, D. Pellitteri-Rosa, F. Pupin, A. Gentilli, S. Tettamanti, L. Gavigioli, L. Racina, V. Maiocchi, P. Galeotti, and M. Fasola. 2010. Photographic identification in reptiles: A matter scales. Amphibia-Reptilia 31:489–502.

Saint Girons, H. 1964. Notes sur l'écologie et la structure des populations de Laticaudinae (Serpentes, Hydrophiidae) in Nouvelle-Calédonie. Terre et Vie 1964 111:185–214. [English translation by I. Fauci, P. A. Gritis, and H. K. Voris published as:

Notes on the ecology and population structure of the Laticaudinae (Serpentes, Hydrophiidae) in New Caledonia. 1990. Bulletin of the Chicago Herpetological Society 25:197–209.]

Saint Girons, H. 1982. Reproductive cycles of male snakes and their relationships with climate and female reproductive cycles. Herpetologica 38:5–16.

Saint Girons, H. 1985. Comparative data on lepidosaurian reproduction and some time tables. Pp. 35–58. In C. Gans and F. Billett (eds.), *Biology of the Reptilia* Vol. 15. *Development A*. John Wiley, New York.

Saleh, M. A. 1997. *Amphibians and Reptiles of Egypt*. Egyptian Environmental Affairs Agency, Department of Nature Conservation, National Biodiversity Unit, Publication no. 6. Cairo, Egypt.

Sanders, H. L. 1968. Marine benthic diversity: A comparative study. American Naturalist 102:243–282.

Sandlund, O. T., K. Hindar, and A. H. D. Brown (eds.). 1992. *Conservation of Biodiversity for Sustainable Development*. Scandinavian University Press, Oslo, Norway.

Sanger, T. J., P. M. Hime, M. A. Johnson, J. Diani, and J. B. Losos. 2008. Laboratory protocols for husbandry and embryo collection of *Anolis* lizards. Herpetological Review 39:58–63.

Sanger, T. J., J. B. Losos, and J. J. Gibson-Brown. 2008. A developmental staging series for the lizard genus *Anolis*: A new system for the integration of evolution, development, and ecology. Journal of Morphology 269:129–137.

Santos, J. C., L. A. Coloma, K. Summers, J. P. Caldwell, R. Ree, and D. C. Cannatella. 2009. Amazonian amphibian diversity is primarily derived from late Miocene Andean lineages. PLoS Biology 7: e1000056. Doi: 10/1371/journal.pbio.1000056.

Sargeant, G. A., D. H. Johnson, and W. E. Berg. 1998. Interpreting carnivore scent-station surveys. Journal of Wildlife Management 62:1235–1245.

Sarti M. L., S. A. Eckert, A. R. Barragan, and N. García T. 1998. Estimation of the nesting population size of the Leatherback Turtle *Dermochelys coriacea* in the Mexican Pacific during 1995–96 nesting season. P. 94. In S. P. Epperly and J. Braun (compilers), *Proceedings of the Seventeenth Annual Sea Turtle Symposium*. NOAA Technical Memorandum NMFS-SEFSC-415. National Marine Fisheries Service, Miami, Florida.

Sarti, L., S. Eckert, P. Dutton, A. Barragan, and N. García. 2000. The current situation of the Leatherback population on the Pacific coast of Mexico and Central America, abundance and distribution of the nestings: An update. Pp. 85–87. In H. J. Kalb and T. Wibbels (compilers), *Proceedings of the Nineteenth Annual Symposium on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFSC-443. National Marine Fisheries Service, Miami, Florida.

Sarre, S. D. 1996. Habitat fragmentation promotes fluctuating asymmetry but not morphological divergence in two geckos. Researches on Population Ecology 38:57–64.

Saul, W. G. 1981. Paraformaldehyde problems. Curation Newsletter 3:1.

Saumure, R. A., B. Freiermuth, J. Jundt, L. Rowlett, and J. Jewell. 2002. A new technique for the safe capture and transport of crocodilians in captivity. Herpetological Review 33:294–296.

Savage, J. M. 2002. *The Amphibians and Reptiles of Costa Rica: A Herpetofauna between Two Continents, between Two Seas*. University of Chicago Press, Chicago, Illinois.

Savarie, P. J., J. A. Shivik, G. C. White, J. C. Hurley, and L. Clark. 2001. Use of acetaminophen for large-scale control of Brown Treesnakes. Journal of Wildlife Management 65:356–365.

Sawaya, R. J., O. A. V. Marques, and M. Martins. 2008. Composition and natural history of a Cerrado snake assemblage at Itirapina, São Paulo State, southeastern Brazil. Biota Neotropica 8:127–149.

Sawyer, J. O., and T. Keeler-Wolf. 1995. *A Manual of California Vegetation*. California Native Plant Society, Sacramento, California.

SCBD (Secretariat of the Convention on Biological Diversity). 2008. Guide to the Global Taxonomy Initiative. CBD Technical Series no. 30.

Schaefer, W. H. 1934. Diagnosis of sex in snakes. Copeia 1934:181.

Schemnitz, S. D. 1996. Capturing and handling wild animals. Pp. 106–124. In T. A. Bookhout (ed.), *Research and Management Techniques for Wildlife and Habitats*. 5th ed. rev. The Wildlife Society, Bethesda, Maryland.

Scherbak, N. N. 1986. Grundzüge einer herpetogeographischen Gliederung der Paläarktis. [Features of herpetological geographic distributions in the Palearctic]. In I. Conferencia Herpetologica Respublicarum Socialisticarum Budapest, 25–29 August 1981. Vertebrata Hungarica 21:227–239.

Schildger, B.-J., and R. Wicker. 1989. Sex determination and clinical examination in reptiles using endoscopy. Herpetological Review 20:9–10.

Schlaepfer, M. A., C. Hoover, and C. K. Dodd, Jr. 2005. Challenges in evaluating the impact of the trade in amphibians and reptiles on wild populations. Bioscience 55:256–264.

Schleich, H. H., W. Kästle, and K. Kabisch. 1996. *Amphibians and Reptiles of North Africa: Biology, Systematics, Field Guide*. Koeltz Scientific, Koenigstein, Germany.

Schlesinger, C. A., and R. Shine. 1994a. Choosing a rock: Perspectives of a bush-rock collector and a saxicolous lizard. Biological Conservation 67:49–56.

Schlesinger, C. A., and R. Shine. 1994b. Selection of diurnal retreat sites by the nocturnal gekkonid lizard *Oedura lesueurii*. Herpetologica 50:156–163.

Schmidt, K. P. 1951. Annotated bibliography of marine ecological relations of living reptiles (except turtles). Marine Life, Occasional Papers 1:47–54.

Schönecker, P. 2008. *Geckos of Madagascar, the Seychelles, Comoros and Mascarene Islands*. Chimaira, Frankfurt am Main, Germany.

Schroeder, B., and S. Murphy. 1999. Population surveys (ground and aerial) on nesting beaches. Pp. 45–53. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, and M. Donnelly (eds.), *Research and Management Techniques for the Conservation of Sea Turtles*. IUCN/SSC Marine Turtle Specialist Group Publication no. 4.

Schroeder, B. A., and N. B. Thompson. 1987. Distribution of the Loggerhead Turtle, *Caretta caretta*, and the Leatherback Turtle, *Dermochelys coriacea*, in the Cape Canaveral, Florida area: Results of aerial surveys. Pp. 45–53. In W. N. Witzell, (ed.), *Ecology of East Florida Sea Turtles. Proceedings of the Cape Canaveral, Florida Sea Turtle Workshop, Miami, Florida, February 26–27, 1985*. NOAA Technical Report NMFS 53. National Marine Fisheries Service, Miami, Florida.

Schuett, G. W., P. J. Fernandez, W. F. Gergits, N. J. Casna, D. Chiszar, H. M. Smith, J. B. Mitton, S. P. Mackessy, R. A. Odum, and M. J. Demlong. 1997. Production of offspring in the absence of males: Evidence for facultative parthenogenesis in bisexual snakes. Herpetological Natural History 5:1–10.

Schuett, G. W., M. Höggren, M. E. Douglas, and H. W. Greene (eds.). 2002. *Biology of the Vipers*. Eagle Mountain Publishing, Eagle Mountain, Utah.

Schumacher, J., H. B. Lillywhite, W. M. Norman, and E. R. Jacobson. 1997. Effects of ketamine HCl on cardiopulmonary function in snakes. Copeia 1997:395–400.

Schwaner, T. D. 1989. A field study of thermoregulation in Black Tiger Snakes (*Notechis ater niger*: Elapidae) on the Franklin Islands, South Australia. Herpetologica 45:393–401.

Schwartz, A., and R. W. Henderson. 1991. *Amphibians and Reptiles of the West Indies: Descriptions, Distributions, and Natural History*. University of Florida Press, Gainesville, Florida.

Schwartz, C. W., and E. R. Schwartz. 1974. The Three-toed Box Turtle in Central Missouri: Its population, home range, and movements. Missouri Department of Conservation, Jefferson City, Missouri, Terrestrial Series 5:1–28.

Scott, G. P., and J. R. Gilbert. 1982. Problems and progress in the US BLM-sponsored CETAP surveys. Reports of the International Whaling Commission 32:587–599.

Scott, J. M., P. J. Heglund, M. L. Morrison, J. B. Haufler, M. G. Raphael, W. A. Wall, and F .B. Samson (eds.). 2002. *Predicting Species Occurrences: Issues of Accuracy and Scale*. Island Press, Washington, DC.

Scott, N. J., Jr. 1976. The abundance and diversity of the herpetofaunas of tropical forest litter. Biotropica 8:41–58.

Scott, N. J., Jr. (ed.). 1982a. *Herpetological Communities*. U.S. Department of the Interior, Fish and Wildlife Service, Wildlife Research Report 13.

Scott, N. J., Jr. 1982b. The herpetofauna of forest litter plots from Cameroon, Africa. Pp. 145–150. In N. J. Scott, Jr. (ed.), *Herpetological Communities*. U.S. Department of the Interior, Fish and Wildlife Service, Wildlife Research Report 13.

Scott, N. J., Jr. 1994. 1. Complete species inventories. Pp. 78–84. In W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster. *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Smithsonian Institution Press, Washington, DC.

Scott, N. J., Jr., and A. L. Aquino-Shuster. 1989. The effects of freezing on formalin preservation of specimens of frogs and snakes. Collection Forum 5:41–46.

Seber, G. A. F. 1982. *The Estimation of Animal Abundance and Related Parameters*. 2nd ed. C. Griffin, London, England.

Seber, G. A. F. 1986. A review of estimating animal abundance. Biometrics 42:267–292.

Seburn, C. N. L., and C. A. Bishop (eds.). 2007. *Ecology, Conservation and Status of Reptiles in Canada*. Herpetological Conservation Vol. 2. Society for the Study of Amphibians and Reptiles, Salt Lake City, Utah.

Secor, S. M. 1994. Ecological significance of movements and activity range for the Sidewinder, *Crotalus cerastes*. Copeia 1994:631–645.

Seebacher, F., and G. C. Grigg. 1997. Patterns of body temperature in wild freshwater crocodiles, *Crocodylus johnstoni*: Thermoregulation versus thermoconformity, seasonal acclimatization, and the effect of social interactions. Copeia 1997:549–557.

Seibert, H. C. 1950. Population density of snakes in an area near Chicago. Copeia 1950:229–230.

Seibert, H. C., and C. W. Hagen, Jr. 1947. Studies on a population of snakes in Illinois. Copeia 1947:6–22.

Seigel, R. A., and N. B. Ford. 1988. A plea for standardization of body size measurements in studies of snake ecology. Herpetological Review 19:9–10.

Seigel, R. A., J. W. Gibbons, and T. K. Lynch. 1995. Temporal changes in reptile populations: Effects of a severe drought on aquatic snakes. Herpetologica 51:424–434.

Seigel, R. A., R. K. Loraine, and J. W. Gibbons. 1995. Reproductive cycles and temporal variation in fecundity in the Black Swamp Snake, *Seminatrix pygaea*. American Midland Naturalist 134:371–377.

Seigel, R. A., C. A. Sheil, and J. S. Doody. 1998. Changes in a population of an endangered rattlesnake *Sistrurus catenatus* following a severe flood. Biological Conservation 83:127–131.

Semlitsch, R. D., K. L. Brown, and J. P. Caldwell. 1981. Habitat utilization, seasonal activity, and population size structure of the Southeastern Crowned Snake *Tantilla coronata*. Herpetologica 37:40–46.

Semlitsch, R. D., J. H. K. Pechmann, and J. W. Gibbons. 1988. Annual emergence of juvenile Mud Snakes (*Farancia abacura*) at aquatic habitats. Copeia 1988:243–245.

Sessions, S. K. 1996. Chromosomes: Molecular cytogenetics. Pp. 121–168. In D. M. Hillis, C. Moritz, and B. K. Mable (eds.), *Molecular Systematics*. Sinauer, Sunderland, Massachusetts.

Setser, K. 2007. Use of anesthesia increases precision of snake length measurements. Herpetological Review 38:409–411.

Sever, D. M., R. A. Stevens, T. J. Ryan, and W. C. Hamlett. 2002. Ultrastructure of the reproductive system of the Black Swamp Snake (*Seminatrix pygaea*). III. Sexual segment of the male kidney. Journal of Morphology 252:238–254.

Sexton, O. J. 1959a. Spatial and temporal movements of a population of the Painted Turtle, *Chrysemys picta marginata* (Agassiz). Ecological Monographs 29:113–140.

Sexton, O. J. 1959b. A method of estimating the age of Painted Turtles for use in demographic studies. Ecology 40:716–718.

Sexton, O. J., H. Heatwole, and D. Knight. 1964. Correlation of microdistribution of some Panamanian reptiles and amphibians with structural organization of the habitat. Caribbean Journal of Science 4:261–295.

Sexton, O. J., and S. R. Hunt 1980. Temperature relationships and movements of snakes (*Elaphe obsoleta, Coluber constrictor*) in a cave hibernaculum. Herpetologica 36:20–26.

Shaffer, H. B., R. N. Fisher, and C. Davidson. 1998. The role of natural history collections in documenting species declines. Trends in Ecology and Evolution 13:27–30.

Shah, B., R. Shine, S. Hudson, and M. Kearney. 2003. Sociality in lizards: Why do Thick-Tailed Geckos (*Nephrurus milii*) aggregate? Behaviour 140(8/9):1039–1052.

Shah, B., R. Shine, S. Hudson, and M. Kearney. 2004. Experimental analysis of retreat-site selection by Thick-Tailed Geckos *Nephrurus milii*. Austral Ecology 29:547–552.

Shannon, C. E., and W. Weaver. 1949. *The Mathematical Theory of Communication*. University of Illinois Press, Urbana, Illinois.

Shapiro, M. D. 2002. Developmental morphology of limb reduction in *Hemiergis* (Squamata: Scincidae): Chondrogenesis, osteogenesis, and heterochrony. Journal of Morphology 254:211–231.

Sharell, R. 1966. *The Tuatara, Lizards and Frogs of New Zealand*. Collins, London, United Kingdom.

Shaw, J. 1987. *John Shaw's Closeups in Nature*. Amphoto, New York.

Shea, G. M., and G. L. Reddacliff. 1986. Ossifications in the hemipenes of varanids. Journal of Herpetology 20:566–568.

Shealy, R. M. 1976. The natural history of the Alabama Map Turtle, *Graptemys pulchra* Baur, in Alabama. Bulletin of the Florida State Museum, Biological Sciences 21:47–111.

Sheehan, D. M., E. Willingham, D. Gaylor, J. M. Bergeron, and D. Crews. 1999. No threshold dose for estradiol-induced sex reversal of turtle embryos: How little is too much? Environmental Health Perspectives 107:155–159.

Sheil, C. A. 2005. Skeletal development of *Macrochelys temminckii* (Reptilia: Testudines: Chelydridae). Journal of Morphology 263:71–106.

Shetty, S., and R. Shine. 2002. Activity patterns of Yellow-lipped Sea Kraits (*Laticauda colubrina*) on a Fijian island. Copeia 2002:77–85.

Shetty, S., and A. Sivasundar. 1998. Using passive integrated transponders to study the ecology of *Laticauda colubrina*. Hamadryad 23:71–76.

Shine, C., N. Shine, R. Shine, and D. Slip. 1988. Use of subcaudal scale anomalies as an aid in recognizing individual snakes. Herpetological Review 19:79–80.

Shine, R. 1977. Reproduction in Australian elapid snakes. I. Testicular cycles and mating seasons. Australian Journal of Zoology 25:647–653.

Shine, R. 1983. Reptilian reproduction modes: The oviparity-viviparity continuum. Herpetologica 39:1–8.

Shine, R. 1991a. *Australian Snakes: A Natural History*. Cornell University Press. Ithaca, New York.

Shine, R. 1991b. Intersexual dietary divergence and the evolution of sexual dimorphism in snakes. American Naturalist 138:103–122.

Shine, R. 1993. Sexual dimorphism in snakes. Pp. 49–86. In R. A. Seigel and J. T. Collins (eds.), *Snakes: Ecology and Behavior.* McGraw-Hill, New York.

Shine R., Ambariyanto, P. S. Harlow, and Mumpuni. 1998. Ecological traits of commercially harvested Water Monitors, *Varanus salvator,* in northern Sumatra. Wildlife Research 25:437–447.

Shine R., Ambariyanto, P. S. Harlow, and Mumpuni. 1999a. Ecological attributes of two commercially-harvested python species in Northern Sumatra. Journal of Herpetology 33:249–257.

Shine, R., Ambariyanto, P. S. Harlow, and Mumpuni. 1999b. Reticulated Pythons in Sumatra: Biology, harvesting and sustainability. Biological Conservation 87:349–357.

Shine, R., and X. Bonnet. 2000. Snakes: A new "model organism" in ecological research? Trends in Ecology and Evolution 15:221–222.

Shine, R., M. J. Elphick, P. S. Harlow, I. T. Moore, M. P. LeMaster, and R. T. Mason. 2001. Movements, mating, and dispersal of Red-Sided Gartersnakes (*Thamnophis sirtalis parietalis*) from a communal den in Manitoba. Copeia 2001:82–91.

Shine R., P. Harlow, Ambariyanto, Boeadi, Mumpuni, and J. S. Keogh. 1998. Monitoring monitors: A biological perspective on the commercial harvesting of Indonesian reptiles. Mertensiella 9:61–68.

Shine R., P. Harlow, J. S. Keogh, and Boeadi. 1995. Biology and commercial utilization of Acrochordid snakes, with special reference to Karung (*Acrochordus javanicus*). Journal of Herpetology 29:352–360.

Shine, R., P. S. Harlow, J. S. Keogh, and Boeadi. 1996. Commercial harvesting of giant lizards: The biology of water monitors *Varanus salvator* in southern Sumatra. Biological Conservation 77:125–134.

Shine, R., P. Harlow, M. P. LeMaster, I. T. Moore, and R. T. Mason. 2000. The transvestite serpent: Why do male garter snakes court (some) other males? Animal Behaviour 59:349–359.

Shine, R., and M. Kearney. 2001. Field studies of reptile thermoregulation: How well do physical models predict operative temperatures? Functional Ecology 15:282–288.

Shine, R., S. Keogh, P. Doughty, and H. Giragossyan. 1998. Costs of reproduction and the evolution of sexual dimorphism in a "flying lizard" *Draco melanopogon* (Agamidae). Journal of Zoology (London) 246:203–213.

Shine, R., M. P. LeMaster, I. T. Moore, M. M. Olsson, and R. T. Mason. 2001. Bumpus in the snake den: Effects of sex, size, and body condition on mortality of Red-Sided Garter Snakes. Evolution 55:598–604.

Shine, R., M. LeMaster, M. Wall, T. Langkilde, and R. Mason. 2004. Why did the snake cross the road? Effects of roads on movement and location of mates by garter snakes (*Thamnophis sirtalis parietalis*). Ecology and Society 9(1)9. http://www.ecologyandsociety.org/vol9/iss1/art9/

Shine, R., M. M. Olsson, I. T. Moore, M. P. LeMaster, and R. T. Mason. 1999. Why do male snakes have longer tails than females? *Proceedings of the Royal Society of London* B 266:2147–2151.

Shine, R., R. N. Reed, S. Shetty, and H. G. Cogger. 2002. Relationships between sexual dimorphism and niche partitioning within a clade of sea-snakes (Laticaudinae). Oecologia 133:45–53.

Shine, R., R. N. Reed, S. Shetty, M. LeMaster, and R. T. Mason. 2002. Reproductive isolating mechanisms between two sympatric sibling species of sea snakes. Evolution 56:1655–1662.

Shively, S. H., and J. F. Jackson. 1985. Factors limiting the upstream distribution of the Sabine Map Turtle. American Midland Naturalist 114:292–303.

Shoop, C. R. 1965. Orientation of *Ambystoma maculatum*: Movements to and from breeding ponds. Science 149:558–559.

Shoop, C. R., T. L. Doty, and N. E. Bray. 1981. Sea turtles in the region between Cape Hatteras and Nova Scotia in 1979. Pp. IX.1–IX.85. In Cetacean and Turtle Assessment Program, University of Rhode Island (CETAP), *A Characterization of Marine Mammals and Turtles in the Mid- and North-Atlantic Areas of the US Outer Continental Shelf, Annual Report for 1979*. Bureau of Land Management, Washington, DC.

Shoop, C. R., and R. D. Kenney. 1992. Seasonal distributions and abundances of loggerhead and leatherback sea turtles in waters of the northeastern United States. Herpetological Monographs 6:43–67.

Shoop, C. R., C. A. Ruckdeschel, and N. B. Thompson. 1985. Sea turtles in the southeast United States: Nesting activity as derived from aerial and ground surveys, 1982. Herpetologica 41:252–259.

Showler, D. A., N. Aldus, and J. Parmenter. 2005. Creating hibernacula for common lizards *Lacerta vivipara,* The Ham, Lowestoft, Suffolk, England. Conservation Evidence 2:96–98.

Shuntov, V. P. 1962. Sea snakes (Hydrophiidae) of Tonking Bay (northern Viet-nam [sic]). Zoologicheskii Zhurnal 41:1203–1209.

Shuntov, V. P. 1966. On the distribution of sea snakes in the South-Chinese Sea and East Indian Ocean. Zoologicheskii Zhurnal 45:1882–1886.

Shuntov, V. P. 1971. Sea snakes on the north Australian Shelf. Ekologiya 4:65–72. [in Russian; English translation by Consultants Bureau, Plenum, New York, 1972.]

Silverman, S., and D. L. Janssen. 1996. Diagnostic imaging. Pp. 258–264. In D. R. Mader (ed.), *Reptile Medicine and Surgery*. Saunders, Philadelphia, Pennsylvania.

Simberloff, D. 1972. Properties of the rarefaction diversity measurement. American Naturalist 106:414–418.

Simmons, J. E. 1987. *Herpetological Collecting and Collections Management*. Society for the Study of Amphibians and Reptiles, Herpetological Circular no. 16.

Simmons, J. E. 2002. *Herpetological Collecting and Collections Management*. Rev. ed. Society for the Study of Amphibians and Reptiles, Herpetological Circular no. 31.

Simon, C. A., and B. E. Bissinger. 1983. Paint marking lizards: Does the color affect survivorship? Journal of Herpetology 17:184–186.

Simons, E. V., and J. R. Van Horn. 1971. A new procedure for whole-mount alcian blue staining of the cartilaginous skeleton of chicken embryos, adapted to the clearing procedure in potassium hydroxide. Acta Morphologica Neerlando-Scandinavica 8:281–292.

Simpson, E. H. 1949. Measurement of diversity. Nature 163:688.

Sindaco, R., G. Dora, E. Razzetti, and F. Bernini (eds.). 2006. *Atlas of Italian Amphibians and Reptiles*. Societas Herpetologica Italica. Polistampa, Florence, Italy.

Sindaco, R., and V. K. Jeremčenko. 2008. *The Reptiles of the Western Palearctic. 1. Annotated Checklist and Distributional Atlas of the Turtles, Crocodilians, Amphisbaenians and Lizards of Europe, North Africa, Middle East and Central Asia*. Belvedere, Latina, Italy.

Sinervo, B. et al. [25 co-authors]. 2010. Erosion of lizard diversity by climate change and altered thermal niches. Science 328:894–899.

Singer, P. 1975. *Animal Liberation: A New Ethics for our Treatment of Animals*. Avon, New York.

Singh, L. A. K. 1985. Notes on tracking and terrestrial activities of the freshwater turtle *Kachuga tentoria* in River Mahanadi, Orissa. Journal of the Bombay Natural History Society 82:414–417.

Singh, L. A. K. 2000. Interpreting visual signs of the Indian Crocodile. Crocodile Specialist Group Newsletter 19(1):7–9.

Skaar, J., K. Hegg, T. Moe, and K. Smedsrud. 1989. *WMO International Hygrometer Intercomparison (Norway, 1987–1989)*. World Meteorological Organization, Geneva, Switzerland.

Skalski, J. R., and D. S. Robson. 1992. *Techniques for Wildlife Investigations: Design and Analysis of Capture Data*. Academic Press, New York.

Sladky, K. K., V. Miletic, J. Paul-Murphy, M. E. Kinney, R. K. Dallwig, and S. M. Johnson. 2007. Analgesic efficacy and respiratory effects of butorphanol and morphine in turtles. Journal of the American Veterinary Medical Association 230:1356–1362.

Slay, C. K., M. Zani, C. Emmons, E. LaBrecque, B. Pike, S. Kraus, and R. Kenney. 2002. *Early Warning System 1994–2002: Aerial Surveys to Reduce Ship/Whale Collisions in the North Atlantic Right Whale Calving Ground*. Final report, 2002 ed., to U.S. Department of Commerce, NOAA, National Marine Fisheries Service, Center for Coastal Environmental Health and Biomolecular Research, Charleston, South Carolina. Edgerton Research Laboratory, New England Aquarium, Boston, Massachusetts.

Sleeman, J. M., and J. Gaynor. 2000. Sedative and cardiopulmonary effects of medetomidine and reversal with atipamezole in Desert Tortoises (*Gopherus agassizii*). Journal of Zoo and Wildlife Medicine 31:28–35.

Smallwood, K. S. 2001. Linking habitat restoration to meaningful units of animal demography. Restoration Ecology 9:253–261.

Smith, A. B. 1994. *Systematics and the Fossil Record: Documenting Evolutionary Patterns*. Blackwell Scientific, Oxford, England.

Smith, H. M., M. J. Preston, and R. E. Jones. 1989. Oviductal, not oviducal. Anatomical Record 223:446–447.

Smith, L. L., D. A. Steen, J. M. Stober, M. C. Freeman, S. W. Golladay, L. M. Conner, and J. Cochrane. 2006. The vertebrate fauna of Ichauway, Baker County, GA. Southeastern Naturalist 5:599–620.

Smith, M. 1926. *Monograph of the Sea-snakes* (*Hydrophiidae*). British Museum (Natural History), London, England.

Smith, M. A. 1943. *The Fauna of British India, Ceylon and Burma, including the whole of the Indo-Chinese Sub-Region. Reptilia and Amphibia*. Vol. III. *Serpentes*. Taylor and Francis, London, England.

Smith, R., A. Chambers, K. Herpich, T. Tuberville, and J. Berish. 2001. Gopher Tortoise burrow survey methods: External characteristics classification, burrow cameras, and truth. Abstract Pp. 137–138. In The Herpetologists' League and Society for the Study of Amphibians and Reptiles Joint Annual Meetings, 27–31 July 2001, Indianapolis, Indiana.

Smith, R. B., T. D. Tuberville, A. L. Chambers, K. M. Herpich, and J. E. Berish. 2005. Gopher Tortoise burrow surveys: External characteristics, burrow cameras, and truth. Applied Herpetology 2:161–170.

Smith, R. L. 1974. *Ecology and Field Biology*. 2nd ed. Harper Row, New York.

Smolensky, N. L., and L. A. Fitzgerald. 2010. Distance sampling underestimates population densities of dune-dwelling lizards. Journal of Herpetology 44:372–381.

Snover, M. L., A. A. Hohn, and S. A. Macko. 2000. Detecting the precise time at settlement from pelagic to benthic habitats in the Loggerhead Sea Turtle, *Caretta caretta*. P. 174. In H. J. Kalb and T. Wibbels (compilers), *Proceedings of the Nineteenth Annual Symposium on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFSC-443. National Marine Fisheries Service, Miami, Florida.

Soberón M., J., and J. Llorente B. 1993. The use of species accumulation functions for the prediction of species richness. Conservation Biology 7:480–488.

Sokal, R. R., and F. J. Rohlf. 1995. *Biometry*: *The Principles and Practices of Statistics in Biological Research*. 3rd ed. Freeman, New York.

Solórzano, A. 2004. *Snakes of Costa Rica: Distribution, Taxonomy, and Natural History*. Instituto Nacional de Biodiversidad (INBio), Santa Domingo de Heredia, Costa Rica.

Somaweera, R., and N. Somaweera. 2009. *Lizards of Sri Lanka: A Colour Guide with Field Keys*. Chimaira, Frankfurt am Main, Germany.

Soulé, M. E. (ed.). 1987. *Viable Populations for Conservation*. Cambridge University Press, Cambridge, United Kingdom.

Southwood, T. R. E., and P. A. Henderson. 2000. *Ecological Methods*. 3rd ed. Blackwell Science, Oxford, England.

Souza, F. L., and A. S. Abe. 1995. Observations on feeding habits of *Hydromedusa maximiliani* (Testudines: Chelidae) in southeastern Brazil. Chelonian Conservation and Biology 1:320–322.

Souza, F. L., and A. S. Abe. 1997. Population structure, activity, and conservation of the neotropical freshwater turtle, *Hydromedusa* *maximiliani,* in Brazil. Chelonian Conservation and Biology 2:521–525.

Spawls, S., K. Howell, R. Drewes, and J. Ashe. 2002. *A Field Guide to the Reptiles of East Africa*: *Kenya, Tanzania, Uganda, Rwanda and Burundi*. Academic Press, San Diego, California.

Spellerberg, I. F. 1998. Ecological effects of roads and traffic: A literature review. Global Ecology and Biogeography Letters 7:317–333.

Spellerberg, I. F., and I. Prestt. 1978. Marking snakes, Pp. 133–141. In B. Stonehouse (ed.), *Animal Marking*: *Recognition Marking of Animals in Research*. University Park Press, Baltimore, Maryland.

Spotila, J. R., A. E. Dunham, A. J. Leslie, A. C. Steyermark, P. T. Plotkin, and F. V. Paladino. 1996. Worldwide population decline of *Dermochelys coriacea*: Are Leatherback Turtles going extinct? Chelonian Conservation and Biology 2:209–222.

St. Clair, R., P. T. Gregory, and J. M. Macartney. 1994. How do sexual differences in growth and maturation interact to determine size in northern and southern painted turtles? Canadian Journal of Zoology 72:1436–1443.

Stafford, P. J., and J. R. Meyer. 2000. *A Guide to the Reptiles of Belize*. Academic Press, San Diego, California.

Stahl, S. J. 1995. Use of telazol in Eastern Box Turtles (*Terrapene carolina carolina*). Bulletin of the Association of Reptilian and Amphibian Veterinarians 5:4.

Stamps, J. A. 1973. Displays and social organization in female *Anolis aeneus*. Copeia 1973:264–272.

Stanley, T. R., and K. P. Burnham. 1999. A closure test for time-specific capture-recapture data. Environmental and Ecological Statistics 6:197–209.

Starace, F. 1998. *Guide des Serpents et Amphisbènes de Guyane Française*. IBIS Rouge, Guadeloupe, Guyane.

Stark, R. C., and S. F. Fox. 2000. Use of fluorescent powder to track horned lizards. Herpetological Review 31:230–231.

Stark, R. C., S. F. Fox, and D. M. Leslie, Jr. 2005. Male Texas Horned Lizards increase daily movements and area covered in spring: A mate searching strategy? Journal of Herpetology 39:169–173.

Steadman, D. W., J. P. White, and J. Allen. 1999. Prehistoric birds from New Ireland, Papua New Guinea: Extinctions on a large Melanesian island. *Proceedings of the National Academy of Sciences (USA)* 96:2563–2568.

Stebbins, R. C. 1954. *Amphibians and Reptiles of Western North America*. McGraw-Hill, New York.

Stebbins, R. C. 1985. *A Field Guide to Western Reptiles and Amphibians*. 2nd ed., rev. Houghton Mifflin, Boston, Massachusetts.

Stebbins, R. C. 2003. *A Field Guide to Western Reptiles and Amphibians*. 3rd ed. Houghton Mifflin, New York.

Steen, D. A., G. J. Miller, S. C. Sterrett, and L. L. Smith. 2007. *Masticophis flagellum flagellum* (Eastern Coachwhip). Growth and movement. Herpetological Review 38:90.

Stewart, P. 1988. Techniques for photographic size estimations of crocodilians. Herpetological Review 19:80–82.

Stork, N. E. 1993. How many species are there? Biodiversity and Conservation 2:215–232.

Stork, N. E., and P. M. Hammond. 1997. Sampling arthropods from tree-crowns by fogging with knockdown insecticides: Lessons from studies of oak tree beetle assemblages in Richmond Park (UK). Pp. 1–26. In N. E. Stork, J. Adis, and R. E. Didham (eds.), *Canopy Arthropods*. Chapman Hall, London, England.

Storm, R. M., and R. A. Pimental. 1954. A method for studying amphibian breeding populations. Herpetologica 10:161–166.

Strong, D., B. Leatherman, and B. H. Brattstrom. 1993. Two new simple methods for catching small fast lizards. Herpetological Review 24:22–23.

Stuebing, R. B, and R. F. Inger. 1999. *A Field Guide to the Snakes of Borneo*. Natural History Publications (Borneo), Kota Kinabalu, Sabah, Malaysia.

Stuebing, R., and H. K. Voris. 1990. Relative abundance of marine snakes on the west coast of Sabah, Malaysia. Journal of Herpetology 24:201–202.

Suarez, A. V., J. Q. Richmond, and T. J. Case. 2000. Prey selection in horned lizards following the invasion of Argentine ants in southern California. Ecological Applications 10:711–725.

Suarez, A. V., and N. D. Tsutsui. 2004. The value of museum collections for research and society. Bioscience 54:66–74.

Sugerman, R. A., and R. A. Hacker. 1980. Observer effects on collared lizards. Journal of Herpetology 14:188–190.

Sugihara, G. 1980. Minimal community structure: An explanation of species abundance patterns. American Naturalist 116:770–787.

Sullivan, B. K. 1981a. Distribution and relative abundance of snakes along a transect in California. Journal of Herpetology 15:247–248.

Sullivan, B. K. 1981b. Observed differences in body temperature and associated behavior of four snake species. Journal of Herpetology 15:245–246.

Sullivan, B. K. 2000. Long-term shifts in snake populations: A California site revisited. Biological Conservation 94:321–325.

Sun, L., R. Shine, Z. Debi, and T. Zhengren. 2001. Biotic and abiotic influences on activity patterns of insular pit-vipers (*Gloydius shedaoensis*, Viperidae) from north-eastern China. Biological Conservation 97:387–398.

Sutherland, W. J. (ed.). 1996. *Ecological Census Techniques: A Handbook*. Cambridge University Press, Cambridge, England.

Sutton, P. E., H. R. Mushinsky, and E. D. McCoy. 1999. Comparing the use of pitfall drift fences and cover boards for sampling the threatened Sand Skink (*Neoseps reynoldsi*). Herpetological Review 30:149–151.

Suzuki, H. K. 1963. Studies on the osseous system of the slider turtle. Annals of the New York Academy of Sciences 109:351–410.

Swets, J. A. 1988. Measuring the accuracy of diagnostic systems. Science 240:1285–1293.

Swingland, I. R. 1978. Marking reptiles. Pp. 119–132. In B. Stonehouse (ed.), *Animal Marking*: *Recognition Marking of Animals in Research*. University Park Press, Baltimore, Maryland.

Tan, L.-j., Y. Liu, and S.-q. Chen. 2001. A series of stages in the embryonic development of the turtle, *Chinemys reevesii*. Acta Hydrobiologica Sinica 25:605–612. [in Chinese with English abstract].

Tanaka, H., Y. Hayashi, and Y. Wada. 1999. Population density of Habu on the Amami Islands, as estimated by removal methods. Pp. 230–235. In G. H. Rodda, Y. Sawai, D. Chiszar, and H. Tanaka (eds.), *Problem Snake Management: The Habu and the Brown Treesnake*. Comstock, Cornell University Press, Ithaca, New York.

Tanner, B. D. 1990. Automated weather stations. Remote Sensing Reviews 5:73–98.

Taskavak, E., and M. K. Atatür. 1998. Distribution and habitats of the Euphrates Softshell Turtle, *Rafetus euphraticus,* in southeastern Anatolia, Turkey, with observations on biology and factors endangering its survival. Chelonian Conservation and Biology 3:20–30.

Taub, A. M. 1962. The use of paraformaldehyde as a field preservative. Copeia 1962:209–210.

Taulman, J. F., and L. W. Robbins. 1996. Recent range expansion and distributional limits of the Nine-Banded Armadillo (*Dasypus novemcinctus*) in the United States. Journal of Biogeography 23:635–648.

Taylor, D., and L. Winder. 1997. The use of imitation sand lizards to assess the accuracy of visual surveying techniques. Herpetological Journal 7:119–121.

Taylor, E. H. 1953. A review of the lizards of Ceylon. University of Kansas Science Bulletin. Vol. 35, (12):1525–1585.

Taylor, E. H. 1956. A review of the lizards of Costa Rica. University of Kansas Science Bulletin 38(1):3–322.

Taylor, E. H. 1963. The lizards of Thailand. University of Kansas Science Bulletin 44(14):687–1077.

Taylor, W. R. 1977. Observations on specimen fixation. *Proceedings of the Biological Society of Washington* 90:753–763.

Terranella, A. C., L. Ganz, and J. J. Ebersole. 1999. Western harvester ants prefer nest sites near roads and trails. Southwestern Naturalist 44:382–384.

Teska, W. R. 1976. Terrestrial movements of the Mud Turtle *Kinosternon scorpioides* in Costa Rica. Copeia 1976:579–580.

Thirakhupt, K., and P. P. van Dijk. 1994. Species diversity and conservation of turtles of western Thailand. Natural History Bulletin of the Siam Society 42:207–259.

Thomas, L., and C. J. Krebs. 1997. A review of statistical power analysis software. Bulletin of the Ecological Society of America 78:126–139.

Thomas, L., S. T. Buckland, E. A. Rexstad, J. L. Laake, S. Strindberg, S. L. Hedley, J. R. B. Bishop, T. A. Marques, and K. P. Burnham. 2010. Distance software: Design and analysis of distance sampling surveys for estimating population size. Journal of Applied Ecology 47:5–14. DOI: 10.1111/j.1365-2664.2009.01737.x

Thomas, R. B., D. W. Beckman, K. Thompson, K. A. Buhlmann, J. W. Gibbons, and D. L. Moll. 1997. Estimation of age for *Trachemys scripta* and *Deirochelys reticularia* by counting annual growth layers in claws. Copeia 1997:842–845.

Thompson, D., and A. R. Hiby. 1985. The use of scale binoculars for distance estimation and a time-lapse camera for angle estimation during the 1983/84 IDCR Minke Whale assessment cruise. Reports of the International Whaling Commission 35:309–314.

Thompson, F. R., III, W. Dijak, and D. E. Burhans. 1999. Video identification of predators at songbird nests in old fields. Auk 116:259–264.

Thompson, S. K. 1991. Adaptive cluster sampling: Designs with primary and secondary units. Biometrics 47:1103–1115.

Thompson, S. K., and G. A. F. Seber. 1996. *Adaptive Sampling.* Wiley, New York.

Thompson, W. L., G. C. White, and C. Gowan. 1998. *Monitoring Vertebrate Populations*. Academic Press, San Diego, California.

Thorbjarnarson, J. B. 1989. Ecology of the American crocodile, *Crocodyls acutus*. Pp. 228–258. In *Crocodiles: Their Ecology, Management, and Conservation*. Special Publication of the Crocodile Specialist Group of the Species Survival Commission of the International Union for Conservation of Nature and Natural Resources, Gland, Switzerland.

Thorbjarnarson, J. (compiler). 1992. *Crocodiles. An action Plan for Their Conservation*. International Union for Conservation of Nature and Natural Resources. Gland, Switzerland.

Thorbjarnarson, J. B. 1993. Diet of the Spectacled Caiman (*Caiman crocodilus*) in the central Venezuelan llanos. Herpetologica 49:108–117.

Thorbjarnarson, J. B. 1996. Reproductive characteristics of the order Crocodylia. Herpetologica 52:8–24.

Thorbjarnarson, J., R. Rodríguez Soberón, M. Alonso Tabet, M. Ramos Targarona, and R. Da Silveira. 2000. On the use of camera traps to study crocodilian nesting behavior. Crocodile Specialist Group Newsletter 19(3):17–18.

Throckmorton, G. S. 1981. Ketamine hydrochloride as an anesthetic agent for lizard surgery. Copeia 1981:241–243.

Tikader, B. K., and R. C. Sharma. 1985. *Handbook: Indian Testudines*. Zoological Survey of India, Calcutta, India.

Tinkle, D. W. 1958. The systematics and ecology of the *Sternothaerus carinatus* complex (Testudinata, Chelydridae). Tulane Studies in Zoology 6:1–56.

Tinkle, D. W. 1967. The life and demography of the Side-blotched Lizard, *Uta stansburiana*. Miscellaneous Publications Museum Zoology, University of Michigan 132:1–182.

Tinkle, D. W. 1979. Long-term field studies. BioScience 29:717.

Todd, B. D., and K. M. Andrews. 2008. Response of a reptile guild to forest harvesting. Conservation Biology 22:753–761.

Tokita, M., and S. Kuratani. 2001. Normal embryonic stages of the Chinese softshelled turtle *Pelodiscus sinensis* (Trionychidae). Zoological Science 18:705–716.

Towns, D. R. 1991. Response of lizard assemblages in the Mercury Islands, New Zealand, to removal of an introduced rodent: The Kiore (*Rattus exulans*). Journal of the Royal Society of New Zealand 21:119–136.

Tracy, C. R., and C. R. Tracy. 1995. Estimating age of Desert Tortoises (*Gopherus agassizii*) from scute rings. Copeia 1995:964–966.

Trombulak, S. C., and C. A. Frissell. 2000. Review of ecological effects of roads on terrestrial and aquatic communities. Conservation Biology 14:18–30.

Tu, A. T. 1974. Sea snake investigation in the Gulf of Thailand. Journal of Herpetology 8:201–210.

Tu, M. C., S. C. Fong, and K. Y. Lue. 1990. Reproductive biology of the sea snake, *Laticauda semifasciata,* in Taiwan. Journal of Herpetology 24:119–126.

Tu, M.-C., and Y. Su. 1991. The aggressiveness of the sea snake *Laticauda semifasciata* in Taiwan. Bulletin of the Institute of Zoology, Academia Sinica 30:55–58.

Tuberville, T. D., and V. J. Burke. 1994. Do flag markers attract turtle nest predators? Journal of Herpetology 28:514–516.

Tucker, A. D. 1997. Validation of skeletochronology to determine age of freshwater crocodiles (*Crocodylus johnstoni*). Marine and Freshwater Research 48:343–351.

Tucker, A. D., D. Broderick, and L. Kampe. 2001. Age estimation of *Eretmochelys imbricata* by schlerochronology of carapacial scutes. Chelonian Conservation and Biology 4:219–222.

Tucker, A. D., N. N. FitzSimmons, and C. J. Limpus. 1996. Conservation implications of internesting habitat use by Loggerhead Turtles *Caretta caretta* in Woongarra Marine Park, Queensland, Australia. Pacific Conservation Biology 2:157–166.

Tucker, A. D., J. W. Gibbons, and J. L. Greene. 2001. Estimates of adult survival and migration for Diamondback Terrapins: Conservation insight from local extirpation within a metapopulation. Canadian Journal of Zoology 79:2199–2209.

Tucker, A. D., and C. J. Limpus. 1997. Assessment of reproductive status in Australian freshwater crocodiles (*Crocodylus johnstoni*) by ultrasound imaging. Copeia 1997:851–857.

Tucker, J. K. 1995. Notes on road-killed snakes and their implications on habitat modification due to summer flooding on the Mississippi River in west central Illinois. Transactions of the Illinois State Academy of Science 88:61–71.

Tuljapurkar, S. D., and S. H. Orzack. 1980. Population dynamics in variable environments I. Long-run growth rates and extinction. Theoretical Population Biology 18:314–342.

Turner, F. B., and P. A. Medica. 1982. The distribution and abundance of the Flat-tailed Horned Lizard (*Phrynosoma mcallii*). Copeia 1982:815–823.

Tuttle, S. E., and D. M. Carroll. 2005. Movements and behavior of hatchling Wood Turtles (*Glyptemys insculpta*). Northeastern Naturalist 12:331–348.

Tyre, A. J., B. Tenhumberg, S. A. Field, D. Niejalke, K. Parris, and H. P. Possingham. 2003. Improving precision and reducing bias in biological surveys: Estimating false-negative error rates. Ecological Applications 13:1790–1801.

Uetz, P. 2010. The original descriptions of reptiles. Zootaxa 2334:59–68.

Uetz, P., and collaborators. 2011. The Reptile Database. http://www.reptile-database.org/

Ultsch, G. R., T. E. Graham, and C. E. Crocker. 2000. An aggregation of overwintering Leopard Frogs, *Rana pipiens,* and Common Map Turtles, *Graptemys geographica,* in northern Vermont. Canadian Field-Naturalist 114:314–315.

U.S. National Research Council, Committee on Sea Turtle Conservation. 1990. *Decline of the Sea Turtles: Causes and Prevention*. National Academy Press, Washington, DC.

Ussher, G. T. 1999. Method for attaching radio transmitters to medium-sized reptiles: Trials on Tuatara (*Sphenodon punctatus*). Herpetological Review 30:151–152.

Vac, M. H., L. M. Verdade, C. F. Meirelles, R. E. Larsen, F. Michelotti, M. C. Rangel, C. Z. Salem, and A. Lavorenti. 1992. Ultrasound evaluation of the follicle development in adult female Broad-Nosed Caiman (*Caiman latirostris*). Pp. 176–183. In *Crocodiles: Proceedings of the 11th Working Meeting of the Crocodile Specialist Group of the Species Survival Commission of IUCN - The World Conservation Union.* Vol. 2. IUCN-The World Conservation Union, Gland, Switzerland.

Valenzuela, N. 2001. Genetic differentiation among nesting beaches in the highly migratory Giant River Turtle (*Podocnemis expansa*) from Colombia. Herpetologica 57:48–57.

Valenzuela, N., and V. Lance (eds.). 2004. *Temperature-Dependent Sex Determination in Vertebrates*. Smithsonian Books, Washington, DC.

Valenzuela, N., A. LeClere, and T. Shikano. 2006. Comparative gene expression of steroidogenic factor 1 in *Chrysemys picta* and *Apalone mutica* turtles with temperature-dependent and genotypic sex determination. Evolution and Development 8:424–432.

Valverde, R. A., and C. E. Gates. 1999. Population surveys on mass nesting beaches. Pp. 56–60. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, and M. Donnelly (eds.), *Research and Management Techniques for the Conservation of Sea Turtles.* IUCN/SSC Marine Turtle Specialist Group Publication no. 4.

van Dijk, P. P., B. L. Stuart, and A. G. J. Rhodin (eds.). 2000. *Asian Turtle Trade: Proceedings of a Workshop on Conservation and Trade of Freshwater Turtles and Tortoises in Asia, Phnom Penh, Cambodia, 1–4 December 1999.* Chelonian Research Monographs no. 2.

Van Horne, B. 1983. Density as a misleading indicator of habitat quality. Journal of Wildlife Management 47:893–901.

van Schaik, C. P., and M. Griffiths. 1996. Activity periods of Indonesian rain forest mammals. Biotropica 28:105–112.

Vane-Wright, R. I., C. J. Humphries, and P. H. Williams. 1991. What to protect? – Systematics and the agony of choice. Biological Conservation 55:235–254.

Vane-Wright, R. I., C. R. Smith, and I. J. Kitching. 1994. Systematic assessment of taxic diversity by summation. Pp. 309–326. In P. L.

Forey, C. J. Humphries, and R. I. Vane-Wright (eds.), *Systematics and Conservation Evaluation*. Systematics Association Special Volume 50. Clarendon, Oxford University Press, Oxford, England.

Vargas, G. A., K. L. Krakauer, J. L. Egremy-Hernandez, and M. J. McCoid. 2000. Sticky trapping and lizard survivorship. Herpetological Review 31:23.

Vega-López, A. A., and T. Alvarez S. 1992. La herpetofauna de los volcanes Popocatepetl e Iztaccihuatl. Acta Zoologica Mexicana 51:1–128.

Velhagen, W. R. A. 1995. A Comparative Study of Cranial Development in the Thamnophiine Snakes (Serpentes: Colubridae). Unpubl. Ph.D. dissert. Duke University, Durham, North Carolina.

Velhagen, W. A., and A. H. Savitzky. 1998. Evolution of embryonic growth in thamnophiine snakes. Copeia 1998:549–558

VerCauteren, K. C., M. J. Pipas, and J. Bourassa. 2002. Application of burrow cameras in wildlife damage research. Pp. 17–20. In R. M. Tim and R. H. Schmidt (eds.), *Proceedings, 20th Vertebrate Pest Conference*. University of California, Davis, California.

Verdade, L. M., and F. Sarkis. 1998. Age at first reproduction in captive *Caiman latirostris* (Broad-snouted Caiman). Herpetological Review 29:227–228.

Vetas, B. 1951. Temperatures of entrance and emergence. In Symposium: *A Snake Den in Tooele County, Utah*. Herpetologica 7:15–20.

Vetter, H. 2004a. *Turtles of the World*. Vol. 1. *Africa, Europe and Western Asia*. Chimaira, Frankfurt am Main, Germany.

Vetter, H. 2004b. *Turtles of the World*. Vol. 2. *North America*. Chimaira, Frankfurt am Main, Germany.

Vetter, H. 2005. *Turtles of the World*. Vol. 3. *Central and South America*. Chimaira, Frankfurt am Main, Germany.

Vetter, H., and P. P. van Dijk. 2006. *Turtles of the World*. Vol. 4. *East and South Asia*. Chimaira, Frankfurt am Main, Germany.

Vickaryous, M. K., and B. K. Hall. 2008. Development of the dermal skeleton in *Alligator mississippiensis* (Archosauria, Crocodylia) with comments on the homology of osteoderms. Journal of Morphology 269:398–422.

Viitanen, P. 1967. Hibernation and seasonal movements of the viper, *Vipera berus berus* (L.), in southern Finland. Annales Zoologici Fennici 4:472–546.

Vilella, F. J. 1998. Biology of the mongoose (*Herpestes javanicus*) in a rain forest of Puerto Rico. Biotropica 30:120–125.

Vitt, L. J., T. C. S. Avila-Peres, P. A. Zani, and M. C. Espósito. 2002. Life in shade: The ecology of *Anolis trachyderma* (Squamata: Polychrotidae) in Amazonian Ecuador and Brazil, with comparisons to ecologically similar anoles. Copeia 2002:275–286.

Vitt, L. J., and J. P. Caldwell. 2009. *Herpetology*. 3rd ed. Elsevier, Academic Press, Burlington, Massachusetts.

Vitt, L. J., and C. M. de Carvalho. 1992. Life in the trees: The ecology and life history of *Kentropyx striatus* (Teiidae) in the lavrado area of Roraima, Brazil, with comments on the life histories of tropical teiid lizards. Canadian Journal of Zoology 70:1995–2006.

Vitt, L. J., and S. de la Torre. 1996. *A Research Guide to the Lizards of Cuyabeno*. Museo de Zoología, Centro de Biodiversidad y Ambiente, Pontificia Universidad Católica del Ecuador, Quito, Ecuador. Monograph 1.

Vitt, L. J., E. R. Pianka, W. E. Cooper, Jr., and K. Schwenk. 2003. History and the global ecology of squamate reptiles. American Naturalist 162:44–60.

Vitt, L. J., and S. S. Sartorius. 1999. HOBOs, Tidbits and lizard models: The utility of electronic devices in field studies of ectotherm thermoregulation. Functional Ecology 13:670–674.

Vogel, G. 2006. *Venomous Snakes of Asia*. Chimaira, Frankfurt am Main, Germany.

Vogt, R. C. 1979. Spring aggregating behavior of Painted Turtles *Chrysemys picta* (Reptilia, Testudines, Testudinidae). Journal of Herpetology 13:363–365.

Vogt, R. C. 1980a. Natural history of the map turtles *Graptemys pseudogeographica* and *G. ouachitensis* in Wisconsin. Tulane Studies in Zoology 22:17–48.

Vogt, R. C. 1980b. New methods for trapping aquatic turtles. Copeia 1980:368–371.

Vogt, R. C. 1987. You *can* set drift fences in the canopy! Herpetological Review 18:13–14.

Vogt, R. C. 1994. Temperature controlled sex determination as a tool for turtle conservation. Chelonian Conservation and Biology 1:159–162.

Vogt, R. C., and J. J. Bull. 1984. Ecology of hatchling sex ratio in map turtles. Ecology 65:582–587.

Vogt, R. C., and R. L. Hine. 1982. Evaluation of techniques for assessment of amphibian and reptile populations in Wisconsin. Pp. 201–217. In N. J. Scott, Jr. (ed.), *Herpetological Communities*. U.S. Department of the Interior, Fish and Wildlife Service, Wildlife Research Report 13.

Vonk, F. J., J. F. Admiraal, K. Jackson, R. Reshef, M. A. G. de Bakker, K. Vanderschoot, I. van den Berge, M. van Atten, E. Burgerhout, A. Beck, P. J. Mirtschin, E. Kochva, F. Witte, B. G. Fry, A. E. Woods, and M. K. Richardson. 2008. Evolutionary origin and development of snake fangs. Nature 454:630–633.

von May, R., and M. A. Donnelly. 2009. Do trails affect relative abundance estimates of rainforest frogs and lizards? Austral Ecology 34:613–620.

Voris, H. K. 1964. Notes on the sea snakes of Sabah. Sabah Society Journal 2:138–141.

Voris, H. K. 1985. Population size estimates for a marine snake (*Enhydrina schistosa*) in Malaysia. Copeia 1985:955–961.

Voris, H. K., and W. B. Jeffries. 1995. Predation on marine snakes: A case for decapods supported by new observations from Thailand. Journal of Tropical Ecology 11:569–576.

Voris, H. K., and D. R. Karns. 1996. Habitat utilization, movements, and activity patterns of *Enhydris plumbea* (Serpentes: Homalopsinae) in a rice paddy wetland in Borneo. Herpetological Natural History 4:111–126.

Voris, H. K., and M. W. Moffett. 1981. Size and proportion relationship between the Beaked Sea Snake and its prey. Biotropica 13:15–19.

Voris, H. K., and J. C. Murphy. 2002. The prey and predators of homalopsine snakes. Journal of Natural History 36:1621–1632.

Voris, H. K., and H. H. Voris. 1995. Commuting on the tropical tides: The life of the Yellow-lipped Sea Krait. Ocean Realm April:57–61.

Vrijenhoek, R. C., R. M. Dawley, C. J. Cole, and J. P. Bogart. 1989. A list of the known unisexual vertebrates. Pp. 19–23. In R. M. Dawley and J. P. Bogart (eds.), *Evolution and Ecology of Unisexual Vertebrates*. New York State Museum Bulletin no. 466.

Waichman, A. V. 1992. An alphanumeric code for toe clipping amphibians and reptiles. Herpetological Review 23:19–21.

Waitkuwait, W. E. 1989. Present knowledge on the West African Slender-snouted Crocodile, *Crocodylus cataphractus* Cuvier 1824 and the West African Dwarf Crocodile, *Osteolaemus tetraspis,* Cope 1861. Pp. 260–275. In *Crocodiles: Their ecology, management, and conservation*. Special Publication of the Crocodile Specialist Group of the Species Survival Commission of the International Union for Conservation of Nature and Natural Resources, Gland, Switzerland.

Walczak, J. T. 1991. A technique for the safe restraint of venomous snakes. Herpetological Review 22:17–18.

Wallace, R. L., and L. V. Diller. 2001. Variation in emergence, egress, and ingress among life-history stages and sexes of *Crotalus viridis oreganus* in northern Idaho. Journal of Herpetology 35:583–589.

Waller, T., P. A. Micucci, and E. Alvarenga. 2007. Conservation biology of the Yellow Anaconda (*Eunectes notaeus*) in northeastern Argentina. Pp. 340–363. In R. W. Henderson and R. Powell (eds.), *Biology of the Boas and Pythons*. Eagle Mountain Publishing, Eagle Mountain, Utah.

Walls, G. Y. 1981. Feeding ecology of the Tuatara (*Sphenodon punctatus*) on Stephens Island, Cook Strait. New Zealand Journal of Ecology 4:89–97.

Walsh, B. 1987. Crocodile capture methods used in the Northern Territory of Australia. Pp. 249–252. In G. J. W. Webb, S. C. Manolis, and P. J. Whitehead (eds.), *Wildlife Management: Crocodiles and Alligators*. Surrey Beatty, Chipping Norton, New South Wales, Australia.

Walters, C. J. 1986. *Adaptive Management of Renewable Resources*. Macmillan, New York.

Wang, J. P., and S. C. Adolph. 1995. Thermoregulatory consequences of transmitter implant surgery in the lizard *Sceloporus occidentalis*. Journal of Herpetology 29:489–493.

Wang, R.-T., J. L. Kubie, and M. Halpern. 1977. Brevital sodium: An effective anesthetic agent for performing surgery on small reptiles. Copeia 1977:738–743.

Wapstra, E. 2005. A simple method to accurately measure small squamate offspring. Herpetological Review 36:138–139.

Ward, F. P., C. J. Hohmann, J. F. Ulrich, and S. E. Hill. 1976. Seasonal microhabitat selections of Spotted Turtles (*Clemmys guttata*) in Maryland elucidated by radioisotope tracking. Herpetologica 32:60–64.

Ward, R., E. G. Zimmerman, and T. L. King. 1990. Multivariate analyses of terrestrial reptilian distribution in Texas: An alternate view. Southwestern Naturalist 35:441–445.

Warkentin, I. G., D. Bickford, N. S. Sodhi, and C. J. A. Bradshaw. 2009. Eating frogs to extinction. Conservation Biology 23:1056–1059.

Warwick, C., F. L. Frye, and J. B. Murphy (eds.). 1995. *Health and Welfare of Captive Reptiles*. Chapman Hall, London, England.

Wassenberg, T. J., J. P. Salini, H. Heatwole, and J. D. Kerr. 1994. Incidental capture of sea-snakes (Hydrophiidae) by prawn trawlers in the Gulf of Carpentaria, Australia. Australian Journal of Marine and Freshwater Research 45:429–443.

Watters, J. L. 2008. Comparison of two diet collection methods in the lizards, *Sceloporus jarrovii* and *Sceloporus virgatus*. Herpetological Review 39:307–310.

Waye, H. L. 1999. Size and age structure of a population of Western Terrestrial Garter Snakes (*Thamnophis elegans*). Copeia 1999:819–823.

Waye, H. L., and P. T. Gregory. 1998. Determining the age of garter snakes (*Thamnophis* spp.) by means of skeletochronology. Canadian Journal of Zoology 76:288–294.

Weatherhead, P. J. 1989. Temporal and thermal aspects of hibernation of Black Rat Snakes (*Elaphe obsoleta*) in Ontario. Canadian Journal of Zoology 67:2332–2335.

Weatherhead, P. J., and F. W. Anderka. 1984. An improved radio transmitter and implantation technique for snakes. Journal of Herpetology 18:264–269.

Weatherhead, P. J., and G. P. Brown. 1996. Measurement versus estimation of condition in snakes. Canadian Journal of Zoology 74:1617–1621.

Webb, G. J. W. 2000. Sustainable use of large reptiles - An introduction to issues, Pp. 413–430. In *Crocodiles: Proceedings of the 15th Working Meeting of the Crocodile Specialist Group of the Species Survival Commission of IUCN – The World Conservation Union*. IUCN- The World Conservation Union, Gland, Switzerland.

Webb, G. J. W., R. Buckworth, and S. C. Manolis. 1983. *Crocodylus johnstoni* in the McKinlay River Area, N. T. III. Growth, movement and the population age structure. Australian Wildlife Research 10:383–401.

Webb, G. J. W., and S. C. Manolis. 1989. *Crocodiles of Australia*. Reed, Frenchs Forest, New South Wales, Australia.

Webb, G. J. W., S. C. Manolis, and G. C. Sack. 1984. Cloacal sexing of hatchling crocodiles. Australian Wildlife Research 11:201–202.

Webb, G. J. W., S. C. Manolis, and P. J. Whitehead (eds.). 1987. *Wildlife Management: Crocodiles and Alligators*. Surrey Beatty and the Conservation Commission of The Northern Territory, Chipping Norton, New South Wales, Australia.

Webb, G. J. W., and H. Messel. 1977. Crocodile capture techniques. Journal of Wildlife Management 41:572–575.

Webb, G. J. W., and H. Messel. 1979. Wariness in *Crocodylus porosus* (Reptilia: Crocodilidae). Australian Wildlife Research 6:227–234.

Webb, G. J. W., and A. M. A. Smith. 1987. Life history parameters, population dynamics and the management of crocodilians. Pp. 199–210. In G. J. W. Webb, S. C. Manolis, and P. J. Whitehead (eds.), *Wildlife Management: Crocodiles and Alligators*. Surrey Beatty, Chipping Norton, New South Wales, Australia.

Webb, G. J. W., P. J. Whitehead, and S. C. Manolis. 1987. Crocodile management in the Northern Territory of Australia. Pp.107–124. In G. J. W. Webb, S. C. Manolis, and P. J. Whitehead (eds.), *Wildlife Management: Crocodiles and Alligators*. Surrey Beatty and the Conservation Commission of The Northern Territory, Chipping Norton, New South Wales, Australia.

Webb, J. K., B. W. Brook, and R. Shine. 2002. Collectors endanger Australia's most threatened snake, the Broad-headed Snake *Hoplocephalus bungaroides*. Oryx 36:170–181.

Webb, J. K., and R. Shine. 1997. A field study of spatial ecology and movements of a threatened snake species, *Hoplocephalus bungaroides*. Biological Conservation 82:203–217.

Webb, J. K., and R. Shine. 1998a. Ecological characteristics of a threatened snake species, *Hoplocephalus bungaroides* (Serpentes, Elapidae). Animal Conservation 1:185–193.

Webb, J. K., and R. Shine. 1998b. Using thermal ecology to predict retreat-site selection by an endangered snake species. Biological Conservation 86:233–242.

Webb, J. K., and R. Shine. 2000. Paving the way for habitat restoration: Can artificial rocks restore degraded habitats of endangered reptiles? Biological Conservation 92:93–99.

Webb, R. G., and J. M. Legler. 1960. A new softshell turtle (genus *Trionyx*) from Coahuila, Mexico. University of Kansas Science Bulletin 40:21–30.

Webb, R. G., W. L. Minckley, and J. E. Craddock. 1963. Remarks on the Coahuilan Box Turtle, *Terrapene coahuila* (Testudines, Emydidae). Southwestern Naturalist 8:89–99.

Weir, J. 1992. The Sweetwater rattlesnake round-up: A case study in environmental ethics. Conservation Biology 6:116–127.

Weir, L. A., J. A. Royle, P. Nanjappa, and R. E. Jung. 2005. Modeling anuran detection and site occupancy on North American Amphibian Monitoring Program (NAAMP) routes in Maryland. Journal of Herpetology 39:627–639.

Wemmer, C., T. H. Kunz, G. Lundie-Jenkins, and W. J. McShea. 1996. Mammalian sign. Pp. 157–176. In D. E. Wilson, F. R. Cole, J. D. Nichols, R. Rudran, and M. S. Foster (eds.). *Measuring and Monitoring Biological Diversity: Standard Methods for Mammals*. Smithsonian Institution Press, Washington, DC.

Wermuth, H., and R. Mertens. 1961. *Schildkröten, Krokodile Brückenechsen*. Fischer, Jena, Germany.

Werneburg, I., and M. R. Sánchez-Villagra. 2009. Timing of organogenesis support [*sic*] basal position of turtles in the amniote tree of life. BMC Evolutionary Biology 9:(82), 9pp.

Werner Y. L. 1971. The ontogenic development of the vertebrae in some gekkonoid lizards. Journal of Morphology 133:41–91.

Wharton, C. H. 1969. The Cottonmouth Moccasin on Sea Horse Key, Florida. Bulletin of the Florida State Museum 14:227–272.

Whitaker, P. B., and R. Shine. 1999. When, where and why do people encounter Australian brownsnakes (*Pseudonaja textilis*: Elapidae)? Wildlife Research 26:675–688.

Whitaker, R. 1987. The management of crocodilians in India. Pp. 63–72. In G. J. W. Webb, S. C. Manolis, and P. J. Whitehead (eds.),*Wildlife Management: Crocodiles and Alligators*. Surrey Beatty and the Conservation Commission of The Northern Territory, Chipping Norton, New South Wales, Australia.

Whitaker, R., and A. Captain. 2004. *Snakes of India, the Field Guide*. Draco, Chennai, India.

Whitaker, R., and Z. Whitaker. 1989a. Ecology of the Mugger Crocodile. Pp. 276–296. In *Crocodiles: Their Ecology, Management, and Conservation*. Special Publication of the Crocodile Specialist Group of the Species Survival Commission of the International Union for Conservation of Nature and Natural Resources, Gland, Switzerland.

Whitaker, R., and Z. Whitaker. 1989b. Status and conservation of the Asian crocodilians. Pp. 297–308. In *Crocodiles: Their Ecology, Management, and Conservation*. Special Publication of the Crocodile Specialist Group of the Species Survival Commission of the International Union for Conservation of Nature and Natural Resources, Gland, Switzerland.

White, D., Jr., and D. Moll. 1992. Restricted diet of the Common Map Turtle *Graptemys geographica* in a Missouri stream. Southwestern Naturalist 37:317–318.

White, G. C. 2000. Population viability analysis: Data requirements and essential analyses. Pp. 288–331. In L. Boitani and T. K. Fuller (eds.), *Research Techniques in Animal Ecology: Controversies and Consequences*. Columbia University Press, New York.

White, G. C., D. R. Anderson, K. P. Burnham, and D. L. Otis. 1982. *Capture-Recapture and Removal Methods for Sampling Closed Populations*. National Environmental Research Park, Los Alamos National Laboratory, Los Alamos, New Mexico Report L A-8787-NERP.

White, G. C., K. P. Burnham, D. L. Otis, and D. R. Anderson. 1978. User's Manual for Program CAPTURE. Utah State University. Press, Logan, Utah.

White, G. C., and R. A. Garrott. 1990. *Analysis of Wildlife Radio-Tracking Data*. Academic Press, San Diego, California.

Whitfield S. M., K. E. Bell, T. Philippi, M. Sasa, F. Bolaños, G. Chaves, J. M. Savage, and M. A. Donnelly. 2007. Amphibian and reptile declines over 35 years at La Selva, Costa Rica. *Proceedings of the National Academy of Sciences (USA)* 104:8352–8356.

Whitford, W. G., and F. M. Creusere. 1977. Seasonal and yearly fluctuations in Chihuahuan desert lizard communities. Herpetologica 33:54–65.

Whiting, M. J. 1998. Increasing lizard capture success using baited glue traps. Herpetological Review 29:34.

Whittaker, R. H. 1972. Evolution and measurement of species diversity. Taxon 21:213–251.

Whittaker, R. H. 1977. Evolution of species diversity in land communities. Pp. 1–67. In M. K. Hecht, W. C. Steere, and B. Wallace (eds.), *Evolutionary Biology* Vol. 10. Plenum, New York.

Whittier, J. M., and R. R. Tokarz. 1992. Physiological regulation of sexual behavior in female reptiles. Pp. 24–69. In C. Gans and D. Crews (eds.), *Biology of the Reptilia* Vol. 18. *Physiology E, Hormones, Brain, and Behavior.* University of Chicago Press, Chicago, Illinois.

Wibbels, T. 1999. Diagnosing the sex of sea turtles in foraging habitats. Pp. 139–143. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, and M. Donnelly (eds.), *Research and Management Techniques for the Conservation of Sea Turtles.* IUCN/SSC Marine Turtle Specialist Group Publication no. 4.

Wibbels, T., J. J. Bull, and D. Crews. 1994. Temperature-dependent sex determination: A mechanistic approach. Journal of Experimental Zoology 270:71–78.

Wibbels, T., D. W. Owens, C. J. Limpus, P. C. Reed, and M. S. Amoss, Jr. 1990. Seasonal changes in serum gonadal steroids associated with migration, mating, and nesting in the Loggerhead Sea Turtle (*Caretta caretta*). General and Comparative Endocrinology 79:154–164.

Wikelski, M., and M. Hau. 1995. Is there an endogenous tidal foraging rhythm in marine iguanas? Journal of Biological Rhythms 10:335–350.

Wikelski, M., and F. Trillmich. 1994. Foraging Strategies of the Galapagos Marine Iguana (*Amblyrhynchus cristatus*): Adapting behavioral rules to ontogenetic size change. Behaviour 128:255–279.

Wilbur, S. R. 1967. Live-trapping North American upland game birds. U.S. Department of Interior, Fish and Wildlife Service, Bureau of Sport fisheries and Wildlife, Special Science Report, Wildlife no. 106, 37 pp.

Wilgers, D. J., and E. A. Horne. 2006. Effects of different burn regimes on tallgrass prairie herpetofaunal species diversity and community composition in the Flint Hills, Kansas. Journal of Herpetology 40:73–84.

Wilhelm, F. 2010. *Geckos of Australia*. Chimaira, Frankfurt am Main, Germany.

Wilhoft, D. C. 1963. Gonadal histology and seasonal changes in the tropical Australian lizard, *Leiolopisma rhomboidalis*. Journal of Morphology 113:185–204.

Wilkinson, P. M. 1994. A walk-through snare design for the live capture of alligators. Pp. 74–76. In *Crocodiles: Proceedings of the 12th Working Meeting of the Crocodile Specialist Group of the Species Survival Commission of IUCN-The World Conservation Union.* Vol. 2. IUCN- The World Conservation Union, Gland, Switzerland.

Wilkinson, P. M., and K. G. Rice. 1996. Hind-foot track length: A method for determining the size of American Alligators. Pp. 429–436. In *Crocodiles: Proceedings of the 13th Working Meeting of the Crocodile Specialist Group of the Species Survival Commission of IUCN-The World Conservation Union.* IUCN-The World Conservation Union, Gland, Switzerland.

Williams, B. K., J. D. Nichols, and M. J. Conroy. 2002. *Analysis and Management of Animal Populations: Modeling, Estimation, and Decision Making.* Academic Press, San Diego, California.

Williams, P. H. 2001. *WORLDMAP.* Natural History Museum, London, England. http://www.naturalhistorymuseum.org.uk/science/projects/worldmap/worldmap/info.htm

Williams, P. H., and C. J. Humphries. 1994. Biodiversity, taxonomic relatedness, and endemism in conservation. Pp. 269–287. In P. L. Forey, C. J. Humphries, and R. I. Vane-Wright (eds.), *Systematics and Conservation Evaluation.* Systematics Association Special Volume 50. Clarendon, Oxford University Press, Oxford, England.

Williams, P. H., and C. J. Humphries. 1996. Comparing character diversity among biotas. Pp. 54–76. In K. J. Gaston (ed.), *Biodiversity: A Biology of Numbers and Difference*. Blackwell Science, Cambridge, Massachusetts.

Williams, P. H., C. J. Humphries, and R. I. Vane-Wright. 1991. Measuring biodiversity: Taxonomic relatedness for conservation priorities. Australian Systematic Botany 4:665–679.

Williams, P. H., R. I. Vane-Wright, and C. J. Humphries. 1993. Measuring biodiversity for choosing conservation areas. Pp. 309–328. In J. LaSalle and I. D. Gauld (eds.), *Hymenoptera and Biodiversity.* CAB International, Wallingford, Oxon, England.

Williams, T. A. 1975. The Niches of *Trionyx spiniferus* and *Trionyx muticus* in Iowa: A Comparative Study. Unpubl. Master's thesis, Drake University, Des Moines, Iowa.

Willis, L., S. T. Threlkeld, and C. C. Carpenter. 1982. Tail loss patterns in *Thamnophis* (Reptilia: Colubridae) and the probable fate of injured individuals. Copeia 1982:98–101.

Willson, J. D., and M. E. Dorcas. 2004a. Aspects of the ecology of small fossorial snakes in the western piedmont of North Carolina. Southeastern Naturalist 3:1–12.

Willson, J. D., and M. E. Dorcas. 2004b. A comparison of aquatic drift fences with traditional funnel trapping as a quantitative method for sampling amphibians. Herpetological Review 35:148–150.

Willson, J. D., C. T. Winne, M. E. Dorcas, and J. W. Gibbons. 2006. Post-drought responses of semi-aquatic snakes inhabiting an isolated wetland: Insights on different strategies for persistence in a dynamic habitat. Wetlands 26:1071–1078.

Wilson, D. E., F. R. Cole, J. D. Nichols, R. Rudran, and M. S. Foster (eds.). 1996. *Measuring and Monitoring Biological Diversity: Standard Methods for Mammals*. Smithsonian Institution Press, Washington, DC.

Wilson, D. S. 1994. Tracking small animals with thread bobbins. Herpetological Review 25:13–14.

Wilson, D. S., H. R. Mushinsky, and E. D. McCoy. 1991. Relationship between Gopher Tortoise body size and burrow width. Herpetological Review 22:122–124.

Wilson, D. S., C. R. Tracy, K. A. Nagy, and D. J. Morafka. 1999. Physical and microhabitat characteristics of burrows used by juvenile Desert Tortoises (*Gopherus agassizii*). Chelonian Conservation and Biology 3:448–453.

Wilson, D. S., C. R. Tracy, and C. R. Tracy. 2003. Estimating age of turtles from growth rings: A critical evaluation of the technique. Herpetologica 59:178–194.

Wilson, E. O. 1988. *Biodiversity.* National Academy Press, Washington, DC. 1988.

Wilson, E. O. 1992. *The Diversity of Life.* Belknap, Harvard University Press, Cambridge, Massachusetts.

Wilson, K. R., and D. R. Anderson. 1985. Evaluation of two density estimators of small mammal population size. Journal of Mammalogy 66:13–21.

Wilson, L. D., J. H. Townsend, and J. D. Johnson (eds.). 2010. *Conservation of Mesoamerican Amphibian and Reptiles*. Eagle Mountain Publishing, Eagle Mountain, Utah.

Wilson, S., and G. Swan. 2003. *Reptiles of Australia*. Princeton University Press, Princeton, New Jersey.

Wilson, T. P. 1994. Ecology of the Spotted Turtle, *Clemmys guttata,* at the Western Range Limit. Unpubl. Master's thesis, Eastern Illinois University, Charleston, Illinois.

Winne, C. T., J. D. Willson, B. D. Todd, K. M. Andrews, and J. W. Gibbons. 2007. Enigmatic decline of a protected population of Eastern Kingsnakes, *Lampropeltis getula,* in South Carolina. Copeia 2007:507–519.

Woelfl, S., and M. Woelfl. 1997. Coyote, *Canis latrans,* visitations to scent stations in southeastern Alberta. Canadian Field-Naturalist 111:200–203.

Woltz, H. W., J. P. Gibbs, and P. K. Ducey. 2008. Road crossing structures for amphibians and reptiles: Informing design through behavioral analysis. Biological Conservation 141:2745–2750.

WMO. 1983. *Guide to Meteorological Instruments and Methods of Observation.* World Meteorological Organization no. 8, 5th ed., Geneva, Switzerland. [2008 = 7th ed. http://www.wmo.int/pages/prog/www/IMOP/publications/CIMO-Guide/CIMO_Guide-7th_Edition-2008.html]

Wood, J. M., A. R. Woodward, S. R. Humphrey, and T. C. Hines. 1985. Night counts as an index of American Alligator population trends. Wildlife Society Bulletin 13:262–273.

Wood, J. R., F. E. Wood, K. H. Critchley, D. E. Wildt, and M. Bush. 1983. Laparoscopy of the Green Sea Turtle, *Chelonia mydas*. British Journal of Herpetology 6:323–327.

Woodbury, A. M. 1951. Introduction–a ten-year study. In Symposium: *A Snake Den in Tooele County, Utah*. Herpetologica 7:4–14.

Woodbury, A. M. 1953. Methods of field study in reptiles. Herpetologica 9:87–92.

Woodbury, A. M. 1956. Uses of marking animals in ecological studies: Marking amphibians and reptiles. Ecology 37:670–674.

Woodbury, A. M., and R. M. Hansen. 1950. A snake den in Tintic Mountains, Utah. Herpetologica 6:66–70.

Woodbury, A. M., and R. Hardy. 1948. Studies of the Desert Tortoise, *Gopherus agassizii*. Ecological Monographs 18:145–200.

Woodward, A. R., and W. R. Marion. 1979. An evaluation of factors affecting night-light counts of alligators. Pp. 291–302. In *Proceedings of the Thirty–second Annual Conference, Southeastern Association of Fish and Wildlife Agencies, 1978*.

Woodward, A. R., and C. T. Moore. 1992. *Alligator Age Determination*. Final Report Study no. 7563. Bureau of Wildlife Research, Division of Wildlife, Florida Game and Fresh Water Fish Commission, Tallahassee, Florida.

World Wildlife Fund. 2010. Wildlife Trade: Reptile Trade FAQs. http://www.worldwildlife.org/what/globalmarkets/wildlifetrade/faqs-reptile.html [accessed December, 2010].

Wright, K., C. Pugh, and L. Walker. 1995. Ultrasonographic sexing of helodermatid lizards. Pp. 239–242. In R. E. Junge (ed.), *Proceedings American Association of Zoo Veterinarians, Wildlife Disease Association, American Association of Wildlife Veterinarians Joint Conference East Lansing, Michigan, August 12–17, 1995*.

Wygoda, M. L. 1979. Terrestrial activity of Striped Mud Turtles, *Kinosternon baurii* (Reptilia, Testudines, Kinosternidae) in west-central Florida. Journal of Herpetology 13:469–480.

Wylie, A. P., A. M. O. Veale, and V. E. Sands. 1968. The chromosomes of the Tuatara. *Proceedings of the University of Otago Medical School* 46:22–23.

Wyneken, J. 2001. *The Anatomy of Sea Turtles*. NOAA Technical Memorandum NMFS-SEFSC-470. National Marine Fisheries Service, Miami, Florida.

Wyneken, J., M. H. Godfrey, and V. Bels (eds). 2008. *Biology of Turtles*. CRC Press, Boca Raton, Florida.

Wynne, K., and M. Schwartz. 1999. *Guide to Marine Mammals and Turtles of the US Atlantic and Gulf of Mexico*. Rhode Island Sea Grant, University of Rhode Island, Narragansett, Rhode Island.

Yabe, T. 1992. Sexual difference in annual activity and home range of the Japanese Pond Turtle, *Mauremys japonica,* assessed by mark-recapture and radio-tracking methods. Japanese Journal of Herpetology 14:191–197.

Yardley, J. 2007. China's turtles, emblems of a crisis. New York Times: December 5, 2007.

Yasuda, T., and N. Arai. 2005. Fine-scale tracking of marine turtles using GPS-Argos PTTs. Zoological Science 22:547–553.

Yntema, C. L. 1964. Procurement and use of turtle embryos for experimental procedures. Anatomical Record 149:577–586.

Yntema, C. L. 1968. A series of stages in the embryonic development of *Chelydra serpentina*. Journal of Morphology 125:219–251.

Yoccoz, N. G., J. D. Nichols, and T. Boulinier. 2001. Monitoring of biological diversity in space and time. Trends in Ecology and Evolution 16:446–453.

Yunger, J. A., R. Brewer, and R. Snook. 1992. A method for decreasing trap mortality of *Sorex*. Canadian Field-Naturalist. 106:249–251.

Zaher, H., F. Gobbi Grazziotin, J. E. Cadle, R. W. Murphy, J. C. de Moura-Leite, and S. L. Bonatto. 2009. Molecular phylogeny of advanced snakes (Serpentes, Caenophidia) with an emphasis on South American Xenodontines: A revised classification and descriptions of new taxa. Papéis Avulsos de Zoologia 49:115–153.

Zani, P. A., and L. J. Vitt. 1995. Techniques for capturing arboreal lizards. Herpetological Review 26:136–137.

Zappalorti, R. T., E. W. Johnson, and Z. Leszczynski. 1983. The ecology of the Northern Pine Snake (*Pituophis melanoleucus melanoleucus*) (Daudin) (Reptilia, Serpentes, Colubridae) in southern New Jersey with special notes on habitat and nesting behavior. Bulletin of the Chicago Herpetological Society 18:57–72.

Zappalorti, R. T., and H. K. Reinert. 1994. Artificial refugia as a habitat-improvement strategy for snake conservation. Pp. 369–375. In J. B. Murphy, K. Adler, and J. T. Collins (eds.), *Captive Management and Conservation of Amphibians and Reptiles*. Contributions to Herpetology Vol. 11. Society for the Study of Amphibians and Reptiles, Ithaca, New York.

Zar, J. H. 1999. *Biostatistical Analysis*. 4th ed. Prentice Hall, Upper Saddle River, New Jersey.

Zehr, D. R. 1962. Stages in the normal development of the common garter snake *Thamnophis sirtalis sirtalis*. Copeia 1962:322–329.

Zhang, Y., M. C. Westfall, K. C. Hermes, and M. E. Dorcas. 2008. Physiological and behavioral control of heating and cooling rates in rubber boas, *Charina bottae*. Journal of Thermal Biology 33:7–11.

Zhou Z., and Z. Jiang. 2004. International trade status and crisis for snake species in China. Conservation Biology 18:1386–1394.

Ziegler, T. 2002. *Die Amphibien und Reptilien eines Tieflandfeuchtwald-Schutzgebietes in Vietnam*. Natur und Tier, Berlin, Germany.

Zug, G. R., M. M. Barber, and J. C. Dudley. 1982. Gonadal histology and reproduction in *Carlia bicarinata* (Scincidae, Sauria, Reptilia) of the Port Moresby area, Papua New Guinea. Herpetologica 38:418–425.

Zug, G. R., and R. E. Glor. 1998. Estimates of age and growth in a population of Green Sea Turtles (*Chelonia mydas*) from the Indian River Lagoon system, Florida: A skeletochronological analysis. Canadian Journal of Zoology 76:1497–1506.

Zug, G. R., S. B. Hedges, and S. Sunkel. 1979. Variation in reproductive parameters of three neotropical snakes, *Coniophanes fissidens, Dipsas catesbyi,* and *Imantodes cenchoa*. Smithsonian Contributions to Zoology 300:1–20.

Zug, G. R., W. Z. Lhon, T. Z. Min, K. Kyaw, T. Thin, H. Win, M. T. D. Nyein, K. Aung, and K. T. Tun. 2001. Durability of silt-fencing for drift-fence arrays at a tropical site. Herpetological Review 32:235–236.

Zug, G. R., and J. F. Parham. 1996. Age and growth in Leatherback Turtles, *Dermochelys coriacea* (Testudines: Dermochelyidae): A skeletochronological analysis. Chelonian Conservation and Biology 2:244–249.

Zug, G. R., and A. S. Rand. 1987. Estimation of age in nesting female *Iguana iguana*: Testing skeletochronology in a tropical lizard. Amphibia-Reptilia 8:237–250.

Zweifel, R. G. 1961. Another method of incubating reptile eggs. Copeia 1961:112–113.

Zweig, C. L., F. J. Mazzotti, K. G. Rice. L. A. Brandt, and C. L. Abercrombie. 2004. Evaluation of field measurements of the American Alligator for use in morphometric studies. Herpetological Review 35:43–44.

Zwickel, F. C. 1980. Use of dogs in wildlife biology. Pp. 531–536. In S. D. Schemnitz (ed.), *Wildlife Management Techniques Manual*. 4th ed. rev. Wildlife Society, Washington, DC.

Zwickel, F. C., and A. Allison. 1983. A back marker for individual identification of small lizards. Herpetological Review 14:82.

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